THE ESSENTIALS OF VIRUS DISEASES

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In Memory of My Father
James N. Meenan, M D

PREFACE

In spite of their obvious importance in present day medicine the literature on virus diseases available to those who are not specialists is still very small Too often the practitioner feels that, in dealing with many of those virus diseases which he encounters in practice, he must work alone Conditioned by the rapid help of public health bacteriology he becomes impatient of the delay at present necessary for the diagnosis of virus infections Such a state of affairs is partly due to the virus workers themselves who, perhaps, do not sufficiently recognize the difficulties of the individual practitioner, nor make him acquainted with their own considerable advances in recent years This has resulted in a vicious circle, for it is only by the closest co operation between the laboratory and the practitioner that the diagnosis and control of virus diseases can be advanced

This book has been written in an attempt to narrow the gap, and also to bring home to the student that the study of viruses is now a science in its own right, and requires more than perfunctory mention in textbooks of bacteriology The epidemiologist may indeed find something of value, but the book is obviously not intended for the laboratory worker The latter will find that some matters of importance to him have been omitted, simply because they are not of equal importance to the others No attempt is made to be exhaustive, the study of viruses is now rapidly evolving and new discoveries constantly being made Some of these are fundamental, others ephemeral, and time may be needed to assess them properly Bibliographies have been omitted in an attempt to keep the book within reasonable limits, unless exhaustive they are of doubtful value, and if required can be readily found by reference to those admirable large works which are available My own debt to them is obvious

My sincere thanks are due to many, but particularly to Dr J D McCormack, doyen of Irish epidemiologists, to my colleagues in St Vincent's Hospital, Dr D K O Donovan and Mr William Doolin, to Dr William Hayes of the Post graduate Medical School, and to Dr M P G Stoker of the University of Cambridge All of these read the manuscript and, by guarding me from errors, leave me in their debt For those that may remain I must myself accept responsibility I am also indebted to Miss Kevans who typed the entire manuscript and to Mr J Foley who drew two of the illustrations for me For the others I must thank the World Health Organization and the Controller of His Majesty's Stationery Office, as indicated in the text My gratitude is also due to Messrs J & A Churchill Ltd, and particularly Mr Rivers, for their kindness, courtesy and, above all, their patience with me in all our dealings

I hope that my wife will consider that the finished work reflects in some way her advice, encouragement and help throughout its writing

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Chapter One

THE CHARACTERISTICS OF VIRUSES

The first demonstration of the existence of viruses was provided in 1898 by Beijerinck when he established that the infectious agent of tobacco mosaic disease was filtrable Even earlier than this, in 1892, Ivanowski had carried out filtration experiments on tobacco mosaic with the same results, but had failed to appreciate the significance of his findings Beijerinck, however, recognized their importance and realized that a new type of infectious agent had been discovered In 1898 also, Loeffler and Froesch showed that the causative agent of foot and mouth disease in animals was filtrable and the virus era may be said to have begun

In the immediately succeeding years many diseases affecting man, including yellow fever, rabies, smallpox and poliomyelitis, were shown to belong to the same group Later, the discovery of bacterial viruses, or bacteriophages, widened the group even further It is now known that virus diseases are widespread and that they affect man, animals, plants and bacteria New agents of the same type and new disease pictures caused by them are constantly being reported

It would be unwise, if not impossible, to go very deeply into the nature of those disease agents which we call by the generic name of viruses Several attempts have been made to present a coherent picture of the whole group and none of them were very successful Its various members are too heterogeneous in their known characteristics to allow the application of any general rules which could be recommended to the student

Of late years viruses have rarely been defined Recent intensive study has given us a hint of all that yet remains to

be discovered about them and shows the difficulty of attempting, with our present knowledge, to fit all the viruses affecting man into any rigid scheme of classification Some years ago one frequently defined them as ultramicroscopic, filtrable agents which could multiply only in the presence of living cells This was an unsatisfactory definition because the first two terms in it are only relative, they depend on the micro scopes and on the filters available Nowadays filters can be constructed which will hold back almost any virus and the electron microscope shows us what most of them look like

At present, the nearest one can get to a definition of the viruses is to regard them as a group of infectious agents, most of which are smaller than bacteria, which will multiply only within living cells This is obviously no more than a working rule There are some viruses which do not appear to be infectious there are others which are larger than the smallest bacteria, but there are none which will multiply in the absence of living cells Claims are made from time to time to have grown viruses in cell free media, but they have never been confirmed, and the more we learn about them the more certain it appears that all viruses need living cells for their growth Unfortunately, even this very strict requirement is not confined to the viruses for it applies also to the rickettsiæ, and, although it is a characteristic of the virus group is not peculiar to it

Most of the difficulties in trying to find a satisfactory definition of viruses arise from the fact that we know far less about some of them than we do about others There are at present so many apparent exceptions to any rule which could be laid down that it appears at times that we know very little about viruses This statement is, indeed, true so far as it goes, but it may encourage the student to fall into the opposite error There are gaps, and large gaps, in our knowledge of viruses—most of them due to technical difficulties—but we really do know quite a considerable amount about them Some have become prone to regard viruses as almost incom prehensible metaphysical abstractions Burnet has summed

up this attitude well when he speaks of the exaltation of the "virus" to some mysterious entity which is greater than the sum total of the attributes of its particles This viewpoint is unfortunate for, whatever else viruses may be or may do, the virus particle can be examined physically, chemically and immunologically and its effects can be studied in man or in experimental animals Each virus disease is caused by virus particles which in most cases can, even with our present methods, be separated from the tissues in which they grow, and which will reproduce the disease if transferred to another susceptible host Koch's postulates may not be strictly fulfilled but we can approach closely enough to them to be reasonably sure of our results

ELEMENTARY BODIES

Virus particles are usually termed elementary bodies, and it is now clear that these particles are the cause of the different virus diseases in the same manner as bacteria are the cause of bacterial disease In some instances, such as measles, no elementary bodies have yet been described, although there is little doubt that measles is a virus disease The technical difficulties of growing such viruses have not yet been mastered In the majority of cases elementary bodies can be obtained in pure suspension and can be more or less easily studied depending on the virus concerned The virus of vaccinia is readily handled, that of poliomyelitis is much more difficult to work with

To carry out any fundamental studies on viruses it is usually necessary to obtain a pure suspension of particles or elementary bodies freed from the cells in which they have multiplied This may be done by the disruption of infected cells by, e g grinding in a mortar or by alternate freezing and thawing The resulting material is then centrifuged at a low speed to remove the cellular debris, and finally at a high speed to throw down the virus particles themselves The deposit, containing the virus, is then resuspended and may be

used for infectivity or chemical tests, or for other examinations such as electron microscopy

Some of the large viruses, e g psittacosis, may also be studied to a limited extent inside the cell, in which a definite life cycle can be demonstrated for them The elementary bodies are found grouped together within the cell in a manner analogous to the grouping of bacteria in colonies By micro manipulation methods these virus colonies or inclusion bodies can be proved to be composed of virus particles While we can visualize them inside the cell, and can free them from it, we do not know how they affect the metabolism of the cell itself Our present methods of study have been evolved primarily for the examination of the virus itself, and not for elucidating the relationship between it and the cell

THE VIRUS AND THE CELL

There are many difficulties involved in proving that elementary bodies are infectious, but, on occasion, it has been demonstrated experimentally in a very convincing manner Ideally, one single elementary body should be shown to be capable of initiating infection This is a very rigid test and, in fact, it is doubtful if it is often fulfilled even under natural conditions In some cases, however, we have come very close to it In a series of experiments with vaccinia virus it was found that one out of every four elementary bodies present in a suspension of the virus could be proved to be infectious This is very convincing evidence, and it is doubtful if one could go much further experimentally

There is only one way of determining whether or not any given elementary body is actually infectious, and that is to implant it into susceptible tissues Even then, should it fail to grow, it does not necessarily mean that the virus particle is at fault Although the particle itself may be active it still must get into the cell in order to grow and thus prove its activity The mechanism of entry is unknown—it may be a function of the particle, or of the cell, or of both If it could

be elucidated we might find means of preventing it, and so would have gone a long way towards the prophylaxis of virus infections In spite of a great volume of work, principally with influenza virus in recent years no great progress has, however, yet been made

Even if the problem were solved for one virus there is no reason to suppose that the solution would necessarily be the same for all of them Viruses differ enormously one from another, and they have only one thing in common which is their need for living cells for survival Their methods of getting into the cell and of multiplying in it once there may not be the same in all cases Because in the final analysis their effects on the cell are always the same—proliferation or degeneration—one is inclined to think that they all affect the cell in the same way There is no reason at present known to us why this should be so We are almost completely ignorant of what happens inside an infected cell of what the virus does there and of how it multiplies

Nor should it be assumed that the virus inside the cell is always the same shape or size as is the elementary body which we regard as the virus outside the cell In some cases it may be but in others the elementary body once inside the cell probably divides into a number of smaller particles Such a procedure has already been suggested for influenza Hoyle has postulated that after the inoculation of influenza virus into the allantoic cavity of the chick embryo it multiplies in a non infective form consisting of smaller particles inside the cells lining the cavity There is a marked difference of opinion between Hoyle and some other workers on this point The theory cannot be regarded as proven but it seems to have much to recommend it

Once inside the cell the virus particle does not merely act as a sponge as it were passively absorbing nourishment Some active process is at work We know that this is so because the virus multiplies within the cell If one particle enters a cell a very much larger number of particles is liberated when it breaks down It may be that the virus particles

ally themselves in some way with some of the normal internal structures of the cell but we have little experimental evidence on this point All that can be said with any certainty at present is that viruses require living cells for their growth and that their action within them is to divert the cellular metabolism to their own benefit In this process they will eventually damage or kill the cell and when a sufficient number of cells is *affected clinical signs of disease become* apparent

There is also some evidence that once a virus particle enters a cell it can prevent any other particle from doing so This is known as the interference phenomenon and is one of considerable academic interest It holds out some hope as a possible method of preventing infection with a virulent virus by the deliberate inoculation of a less virulent one but the practical applications of the idea have not been very intensively studied What the mechanism at work may be is quite unknown It would be easier to understand if we could show some relationship antigenic or otherwise between the virus already inside the cell and the one seeking entry but it has been shown time and again that this is not necessary For instance an intracerebral injection of influenza virus in mice will interfere with the growth of St Louis encephalitis virus when the latter is inoculated subsequently The phenomenon is one of the most interesting aspects of the virus cell relationship but it is little understood

ANTIGENICITY

Virus particles have properties other than infectivity Heated virus will no longer be infective but it can be used in various tests for example as the antigen in a complement fixation test Similarly viruses which have been rendered non infectious by formalin or ultraviolet light may be used in vaccines and will stimulate antibody production

The antigenic properties of viruses are of the greatest importance both in diagnosis and in research By means of

them we have been able to differentiate between strains of viruses which cause the same clinical disease pictures in man or animals Obvious examples are the A and B types of influenza virus, or the two types of equine encephalomyelitis found in the United States As well as that, it is possible to reverse the procedure, and to show that viruses which bear little relationship in their clinical effects may nevertheless be closely related antigenically The viruses of the psittacosis-lymphogranuloma group are examples of this

CHEMICAL AND PHYSICAL PROPERTIES

The chemical and physical properties of viruses, while dependent on the structure of the elementary body, are different again from infectivity or antigenicity.

The chemical characteristics have been very intensively studied and critical analyses made of elementary body suspensions It has been found in these cases that part of the material present could be accounted for as being derived from the host cells Because of the difficulty of obtaining absolutely pure suspensions free from all host tissue contamination, chemical analyses of viruses have at times produced different results in the hands of different workers, due no doubt to the varying degrees of purity of their original material In spite of this the chemical structure of some of the viruses has been determined with a high degree of accuracy Vaccinia virus, for example, has been shown to contain carbohydrate, protein, lipoid, thymonucleic acid, biotin and copper The plant viruses have been much more intensively studied than have the animal viruses, on which little work has yet been done, probably due to the greater complexity of their chemical structure

A considerable amount of work has also been carried out on the physical properties of the virus particle This has been principally concerned with studies of shape and size Where relevant the findings are later referred to in the appropriate sections

NATURE

In a work such as this there is little to be gained by entering deeply into the controversy as to whether viruses are living micro organisms or merely complex chemical entities In the early days the general opinion, as expressed by Green and by Laidlaw, was that viruses were micro organisms which, had undergone "retrograde evolution" They were thought to be derived from bacteria or similar micro organisms which, with the passage of time, had lost some or all of the bio *chemical properties of bacteria—such as the ability to* synthesize from the environment the foodstuffs required for survival In this view all viruses were living organisms but were completely parasitic and dependent on their host cells for growth and survival The smaller the virus the more parasitic it was, and the less likely it would be to have any independent metabolic activity of its own The viruses of the psittacosis lymphogranuloma group which were large in size and which looked like bacteria lent weight to this view

Then, in 1935, Stanley reported the crystallization of tobacco mosaic virus and the whole question was thrown into the melting pot with rather indecent haste As other plant viruses were crystallized in turn it seemed that viruses were just protein molecules It was suggested, and the view gained considerable acceptance, that this held also for the animal viruses In spite of the most assiduous attempts, however, no single animal virus has yet been crystallized and the pendulum seems at present to be swinging back It would be difficult now to find anyone who has worked with the animal viruses who regards them merely as protein molecules As Burnet has said "The time has passed, at least in the case of the animal viruses, when it was necessary to withhold the term organism from a virus It is now some years since anyone has spoken of the viruses as non living protein molecules"

This may be regarded as a working hypothesis for the student or practitioner interested in the nature of these

viruses which affect man and animals Whether or not plant viruses differ essentially in their nature remains to be seen Discussions on the nature of viruses have shown a regrettable tendency to tail off into a definition of terms Many of the articles on this subject have been written by plant virologists or chemists who, in some cases, have rather tended to over simplify the subject

ADAPTATION

It is not always sufficiently appreciated that the viruses which are studied in the laboratory are not identical in all cases with those occurring naturally It is obvious that the chick embryo is not the natural host of influenza virus nor for that matter are any of the experimental animals The virus has been persuaded to grow in a new environment

When viruses are first isolated in the animal or chick embryo they grow poorly and not all the particles which are present in the material inoculated, e g sputum or blood, will multiply in the new type of host Only some of them, probably a very small fraction will be capable of adapting themselves to a different environment The others are unable to do so and will die off From those few particles which multiply a new laboratory strain is produced The ease with which this happens varies from virus to virus, and even from strain to strain In some epidemics it is relatively easy to isolate influenza virus from a high proportion of cases, in others it may be difficult to isolate it at all

On first isolation the relationship between the "natural" strain and the "laboratory" strain is very close, but as time passes and numerous passages or subcultures are made, some of their characteristics may diverge For instance, laboratory strains used for studying the reactions of animals to infection are kept at a high level of virulence, probably far above that found in nature All the conclusions drawn from the behaviour of such a strain may not be equally valid After a sufficient number of egg passages influenza virus becomes

avirulent for man—its natural host In spite of this much of our information regarding its behaviour in nature has been taken directly from the behaviour of such laboratory strains without perhaps sufficient attention being paid to these differences

VARIATION

Allied to the question of adaptation is that of the variation of viruses which is an ever present worry to the epidemiologist It is very probable that the pandemic strain of influenza of 1918–19 was a variant of influenza virus as we know it today Similarly the ordinary strains of influenza A virus with which we were familiar until 1946–47 now seem to have largely disappeared their place being taken by others which are closely related but which do show certain differences Chief amongst these is an antigenic difference which means that one would expect vaccines prepared against the older strains to be of little value against the new arrivals So it has proved in practice There is no way of knowing when such variants may arise to present us with new problems when we think that we have mastered the old

On the credit side of the ledger this property of viruses has been turned to our advantage The two most successful examples of immunization in virus diseases are yellow fever and smallpox vaccinations In both of these a variant of the causative virus as it appears in nature is used for the vaccination The phenomenon cannot be produced at will and a certain amount of luck is needed to obtain it but it represents a very fruitful field for future study

It is obvious that if variants of the different viruses could be produced which were antigenically similar to the parent strain but which on inoculation did not produce the ill effects of the fully virulent virus we would have a very valuable weapon indeed for the control of virus infections For some reason which is not fully understood vaccines containing live virus appear to be more powerful than do those containing inactivated virus but the only live vaccines which

can be used with any confidence are those containing non virulent variants of the naturally occurring virus

CLASSIFICATION

It has long been fashionable to attempt to classify virus diseases by the type of cell which a particular virus attacked For instance poliomyelitis was classified as a neurotropic virus herpes as a dermatropic one and so on From the virologist s point of view such a classification has little to recommend it Poliomyelitis and rabies are both neurotropic viruses but apart from that they cannot be said to have much in common Both the lesions they produce and their methods of spread are entirely different The same arguments apply to the classification together of influenza and psittacosis as pneumotropic viruses The main value that such a classification may have is as a catalogue of the viruses which affect particular sites in the body It is a clinical classification of virus disease rather than one of viruses and completely ignores both the properties of the viruses themselves and their methods of spread From the epidemiological point of view smallpox and influenza should be grouped together as should yellow fever and equine encephalomyelitis If one considers only the properties of the viruses concerned lymphogranuloma inguinale and psittacosis should be included in the one heading If size be taken as a criterion then three such widely different viruses and virus diseases as poliomyelitis louping ill and yellow fever may be grouped together at one end of the scale and vaccinia and lymphogranuloma at the other

This criterion of size is frequently used to distinguish viruses but it is a very unsatisfactory one Taking the group as a whole it will be found that the largest of them are in fact larger than some of the bacteria while at the other end of the scale are found those which are smaller than some protein molecules This is obviously far too wide a range to allow of proper classification The viruses are grouped according to their size in Table I but no attempt is made to classify them on that basis

Taken by and large it is difficult to see how any classification of such a heterogeneous variety of organisms as the viruses can properly be attempted at this stage If there were some clue as to the reason why some viruses showed a particular predilection for certain cells, or if we knew why they *preferred one type of cell in one host and a different kind in* another, we might be able to put such a classification on a firmer footing It may be that the cytologist has not interested himself sufficiently in these problems and that there is a clue to be found from a study of these phenomena which would guide us to the essentials of the virus cell relationship

None the less the virus particle must be studied not only at the cellular level but at all levels before any very clear idea of the virus itself can be gained This is not merely the province of the microbiologist in his laboratory All the *information that can be gained about virus diseases* is relevant to the study of their nature To give an example the failure to vaccinate certain people successfully—a minor and every day occurrence—must be capable of explanation This would have a bearing on the behaviour, and thence on the nature of vaccinia virus Truly there is a wide field in virus studies and every particle of information is of importance It is unfortunate that too few practitioners interest themselves in these problems, because only by co operation amongst all branches of science shall we feel our way to a fuller under standing of the nature of viruses and of the diseases they cause

Table I

	Diameter in mμ
Red blood cell	7000
Staphylococcus aureus	800
Psittacosis	275
Varicella	210
Inclusion conjunctivitis	200
Trachoma	200
Mumps	200
Lymphogranuloma	150
Vaccinia	150
Herpes	125
Rabies	125
Pneumonia virus of mice	125
Lymphocytic choriomeningitis	125
Influenza	100
Newcastle disease of fowl	100
Tobacco mosaic virus	30
Equine encephalomyelitis	25
St Louis encephalitis	25
Hæmocyanin molecule	22
Yellow fever	22
Louping ill	19
Coxsackie virus	19
Foot and mouth disease	10
Poliomyelitis	10

As is mentioned in the text the sizes given for the different viruses may vary according to the method used for estimating them Most of the above figures are based on filtration studies Figures given more recently by the electron microscope appear to be somewhat larger

Chapter Two

METHODS OF STUDY AND THEIR APPLICATION

In this chapter are discussed some of the methods used to study viruses and the diagnostic applications of these methods A number of them can be carried out in relatively small laboratories, others require delicate and expensive equipment and highly trained personnel The study of viruses has attracted not only men with a medical training, but also physicists, chemists and botanists, and all of them have made very *substantial contributions to our knowledge*

MICROSCOPY

Contrary to popular belief the ordinary microscope, using transmitted light, can play a very important part in the study of viruses In some cases the virus itself is not by any means "ultramicroscopic," but can be visualized, in many cases specific inclusion bodies can be readily seen, and in all the lesions caused by the virus may be examined by routine histological methods

Using ordinary light the limit of *visibility* with a microscope is stated to be 0 074 μ
the use of a green filter
of great importance—t
resolution and with transmitted light this is of the order of 0 250 μ That is to say that particles cannot be distinguished *as separate* units unless they are at least of this size

It is, of course, somewhat misleading to consider only the size of the virus A virus particle which has been stained will appear, by virtue of the staining, considerably larger than in

its natural state and is therefore more easily visualized Except in the case of ultraviolet light photography and dark ground illumination, referred to below, all such viruses will naturally be stained before examination On the other hand, viruses differ considerably amongst themselves in their ease of staining, and size is not the only criterion Varicella virus, for instance, is not easily stained while variola and vaccinia, approximately the same size, can be stained by simple methods

The viruses which are most easily seen are those of the psittacosis lymphogranuloma group in which all stages of the life cycle can be followed in stained preparations using ordinary transmitted light Indeed this technique is used diagnostically in certain cases, for example in the differentiation of trachoma from follicular conjunctivitis The elementary body of trachoma measures about 200 mμ and so, after staining it is readily seen

Another very important use of ordinary microscopy for diagnostic purposes is the examination of vesicle fluid for elementary bodies in suspected cases of smallpox In many cases this enables a diagnosis to be made by the laboratory within one hour of the specimen reaching it

Direct microscopy is not, of course, used for measuring the diameter of virus particles as it would not be sufficiently accurate for that purpose Other, more accurate, techniques such as are described later are used for this purpose

Using dark ground illumination the limit of resolution can be reduced further and particles less than 0 25 μ in diameter can be studied by this method It takes advantage of the fact that it is much easier to visualize a bright object against a dark background than it is to see a dark object against a bright one

With more complicated, but still relatively simple, equipment such as ultraviolet light the field can be widened even more As ultraviolet light has a shorter wavelength than ordinary light greater resolution is possible with it The object cannot be viewed directly, and photography, as well as other

special equipment, is needed These drawbacks have meant that the method has not been very widely used in spite of its theoretical advantages

Inclusion Bodies

So far only virus particles or elementary bodies have been considered In many instances, however, viruses give rise to specific "inclusion bodies" inside infected cells These inclusions, using the term in the sense understood by virus workers, vary in size up to 10 μ or more and, once stained, are readily visible under the ordinary microscope. They may be found either in the nucleus or in the cytoplasm In some cases they are "colonies" of the virus, that is made up of numbers of virus particles in various stages of development, and can be proved by micro manipulation techniques to be [illegible]

[illegible] granuloma, are diagnostic of infection, are known to be composed, not of virus, but of cellular material

The question of inclusion bodies is rather a vexed one Many histologists consider them to be of little significance, because what they term 'inclusion bodies" can be seen in suitably stained preparations from conditions which are not caused by viruses They point out that "inclusion bodies" can be seen from time to time in healthy tissues Others go to the opposite extreme and consider an inclusion body of any kind to be diagnostic of virus infection, forgetting that many cellular structures may be stained, particularly if they are degenerate, and then appear as structures quite foreign to the cell

Both statements are altogether too sweeping On the one hand inclusion bodies associated with virus infections are specific structures In some cases, such as vaccinia, these specific inclusions may be found in any kind of tissue infected by the virus, whether it be human, animal or chick embryo In most cases they are composed of virus particles

Frequently these particles are embedded in a matrix which is probably derived from the parasitized cell, and this has given rise to the theory that they may be only cellular reactions to infection There is no evidence whatever for the statement that they arise in their entirety from the cell itself On the other hand many virus diseases, notably influenza, do not produce any specific inclusions at all in infected cells

When the virologist speaks of an inclusion body in connection with a virus disease he has in mind a specific structure, constantly found in association with that disease, and in most cases composed of particles of the virus which is causing the disease With the possible exception of the Gamna Favre bodies he is not considering adventitious structures derived from the cell itself, which are of no significance for the diagnosis of infection

The point at issue at the moment, however, is the recognition of these structures Being of large size they can be readily seen with the microscope, and no special techniques are necessary apart from the ordinary methods used for histological preparations Often they may be seen in sections stained with hæmatoxylin and eosin Stains such as phloxine or eosin, with methylene blue as a counterstain, are widely used The Lipschutz bodies of herpes, or the Guarnieri bodies of variola and vaccinia, can be easily demonstrated by such methods

For the Negri bodies of rabies it is usual to stain smears or sections by either Mann's Laidlaw's or Seller's methods, by which, like many other inclusion bodies, they are eosinophilic in their staining reactions

Phase Contrast Microscopy

Inclusion bodies may also be examined by means of phase contrast microscopy By this method a considerable amount of information regarding the relationship between the inclusion and the rest of the cell in which it is present can be obtained Details which are not visible on ordinary microscopy can be elicited Relatively little work has so far been

reported on this method, but it is a simple technique for studying an inclusion body within a living cell The method may be used with either stained or unstained preparations The latter are obviously preferable, as they allow a closer approach to natural conditions without the shrinkage and other modifications of fine detail which occur during fixing and staining To date, however, no diagnostic use seems to have been found for this technique

Electron Microscopy

Few scientific instruments of recent years have aroused so much interest as the electron microscope By means of it magnifications of up to 100 000 diameters can be obtained and particles of 10 mμ visualized This spectacular increase over ordinary light is due to the fact that the electron rays used in place of light have a wavelength of 0 05 Å U, as compared with 3,600 Å U for ultraviolet light and 4 800 8 000 Å U for white light

The principle of the electron microscope is simple Electron rays are used in place of light rays, and magnetic fields replace the lenses of the ordinary microscope These magnetic fields have the same effect on the electron rays as do the lenses of a microscope on light rays The rays which pass through the object being examined are focused and an image is built up *All examinations must be carried out in a vacuum* because a system of electrons such as has been described can exist only *in vacuo*

Of recent years the technique of "shadowing" the object with metals before its examination has been introduced, and with it beautiful three dimensional pictures can be obtained, which enables the shape of a virus particle to be accurately determined Shadow casting is usually done with gold or chromium The object to be examined is mounted *in vacuo* and the metal is discharged from one or more filaments so placed that their "rays" strike the object at a known angle Advantage is taken of the fact that these always travel in a straight line and so the results can be accurately predicted.

Electron rays cannot be seen by the naked eye and the object must be examined by means of a fluorescent screen or by photography. In practice the screen is used in the search for suitable fields for photography.

The object is mounted on a thin film of collodion or similar material and dried, but not stained, before being placed in the instrument. Once there it is also subjected to a vacuum for examination purposes. This process of drying, fixing and examining in a vacuum is a drawback to the virologist, precluding as it does the examination of virus material in its living state. Apart from this disadvantage it also means that the virus particles which are being examined have been subjected to a number of manipulations which must result in a certain amount of shrinkage and distortion. This occurs even with very minute particles in which the effect is, of course, proportionately greater. More serious still the degree of shrinking which may take place is unpredictable.

It is obvious then that the findings of electron microscopy must be interpreted with some caution. It is a new instrument and is capable of further improvement. None the less these disadvantages, which are technical and so capable of being largely eliminated, should not be allowed to weigh against the use of a most valuable instrument. The beautiful electron microphotographs of influenza virus adsorbed on to chick erythrocytes, which have been produced by Elford and Dawson are witness to its potentialities.

As against this too much should not be expected of it, and its use for diagnostic purposes is strictly limited. Only in one case has it been suggested that it might be of value—in the examination of vesicle fluid from suspected cases of variola.

It can be readily understood why this should be so until more experience is gained. The electron microscope has been principally used for the examination of concentrated preparations of elementary bodies—in other words the operator has known what he was examining. The magnifications produced are so enormous that in mixed materials it would be very difficult to identify virus particles accurately in the midst

extraneous matter Similarly, the procedures of drying and photographing *in vacuo* must of necessity have a marked effect on the material and it is at present impossible to predict these effects with confidence Histological material, specially prepared, has been examined in the electron microscope but this is a very specialized procedure indeed

Amongst the viruses which have been minutely studied are those of vaccinia, which has a rectangular brick shaped structure, influenza viruses which are more or less spherical, and the viruses of the American equine encephalitides which are also spherical The most interesting from the viewpoint of morphology are the bacteriophages, some of which have been shown to be shaped like spermatozoa

ULTRACENTRIFUGATION

Apart from the electron microscope many other physical methods are in frequent use for fundamental studies on the virus particle The centrifuge may not only be used for obtaining concentrated samples of a virus, but by means of it a very considerable amount of information can also be gained indirectly as to the shape and size of the virus

Even though the elementary bodies of the larger viruses may be thrown down by angle centrifuges of the type found in most laboratories, it is obvious that something more efficient is needed in the case of the smaller ones The smaller the particle the greater is the centrifugal force needed to deposit it

Instruments such as the Sharples centrifuge, or the air driven instruments which revolve at 50,000 or 60,000 revolutions per minute are widely used The most powerful yet described is the Svedberg type which is driven by oil turbines With this speeds of up to 160,000 revolutions per minute can be produced

Many of these machines have an optical unit incorporated in them so that photographic records can be made of the sedimentation rate of the particles being examined Apart

from its obvious value of being a simple means of checking the speed at which any particle is thrown down, it also may be of importance in helping to determine whether more than one type of particle is present in the material, e g a contaminating virus When viruses are centrifuged at these high speeds an upper boundary line between the sedimenting particles and the fluid in which they are suspended can be made out by special methods If particles of two different sizes are present in the fluid two boundary lines will be seen If a number of them of varying sizes are present only an indistinct boundary can be perceived

By centrifuging viruses in suspensions of differing densities, saccharose being commonly used, it is possible to ascertain their specific gravity and thus by means of mathematical formulæ their size These indirect methods have not been used so frequently since the introduction of the electron microscope

Apart from these fundamental investigations into virus particle size it is sometimes necessary to separate large quantities of virus, for example from infected allantoic fluid in the production of vaccines The Sharples continuous flow centrifuge is widely used for this type of work as large quantities of material can be dealt with in a short space of time

FILTRATION

In the early days of virus studies one of the distinguishing marks attributed to a virus was that it was filtrable, whereas bacteria in general were not The filters used were of porcelain or earthenware such as the Chamberland, Berkefeld or Mandler types and with them relatively good results were obtained in the investigation of those diseases of man, animals and plants which were thought to be caused by viruses When it came to more critical studies such as determining the actual size of the virus particles the results were not so satisfactory There was a certain unpredictability about them even in those cases in which all that was required

was the removal of contaminating bacteria from a suspension of virus Filtrates which should have had a high titre were found to be non infectious

Apart from its practical disadvantages, such as difficulty in cleaning and liability to flaws, a bacteriological filter is not a mere mechanical sieve Several factors, other than size, influence whether or not a given body will pass through it For instance, the electrical charge on the virus and on the filter, the adsorption of the virus on to aggregates of cell debris or on to the filter itself, the temperature at which the filtration is conducted, the amount of positive or negative pressure employed, and the duration of the experiment will all influence the results of filtration

In recent years collodion membranes or gradocol filters have been introduced by Elford and these approach much more nearly to the ideal of the mechanical sieve They are widely used for critical studies of particle size In their preparation nitrocellulose is dissolved in a mixture of alcohol, ether and acetone By the addition of water or acetic acid in measured volumes, the average pore diameter of the membrane can be increased or decreased as desired Any diameter within a wide range can be produced, varying down to as small as 10 mμ

These gradocol membranes are not used as routine filters for the separation of virus from bacteriologically infected materials Where this is done at all by filtration nowadays the Seitz asbestos disc type is most commonly used, but since the advent of chemotherapy filtration for this purpose has lost most of its value Infected material, even if grossly con taminated with bacteria, can be inoculated into chick embryos without any ill effects by the addition of sulphon amides, penicillin or streptomycin, or a mixture of the two latter, to the inoculum Fæcal material can now be injected into the amniotic cavity if streptomycin is used As these antibiotics do not affect viruses they can be employed with perfect safety for isolation purposes, and the amount of virus present in the primary material, which may be quite small

is not diminished by being passed through filters to remove the bacteria

SEROLOGICAL REACTIONS

The immunological characteristics of viruses are analogous to those of bacteria and many of the serological phenomena which have been demonstrated with bacteria may with certain modifications of technique be applied to the study of viruses Agglutination precipitation complement fixation and virus neutralization tests may all be used and in recent years with increasing knowledge many more diseases of virus origin are being diagnosed by serological methods

It is obvious that in the case of viruses difficulties immediately arise The principal one is the preparation of suitably specific antigens for testing purposes Viruses grow only in living cells and in order to obtain pure suspensions for *in vitro* tests it is necessary to separate the cells and all tissue materials from the virus as far as possible This can be attempted by differential centrifugation or by other means but it is almost impossible to eliminate all traces of protein derived from the tissue cells A special control must therefore be included in most tests consisting of an extract of normal cells from the same location which is prepared exactly as the infected material For example in complement fixation tests using antigens derived from infected chick embryo yolk sac a control of uninfected yolk sac is always included

It may be mentioned that in the serological diagnosis of any virus infection at least two specimens of blood should be examined The demonstration of antibodies to a virus in one specimen of blood is no proof that the disease under investigation was in fact caused by that virus They may have come from an earlier attack One of the characteristics of most virus infections is the long lived immunity which an attack produces and this immunity is usually due to the continued presence of antibody in the blood It is therefore necessary to have at least two specimens of blood for examination one

collected early in the disease and the other at a later period when antibodies have had time to appear Should there be a significant difference in the titre of the two specimens a diagnosis of recent infection can be made

Virus Neutralization Tests

The serological tests available may be used either for the diagnosis of infection with a virus or for the identification of a recently discovered strain The test most commonly employed for this latter is the virus neutralization test, in which anti sera to known strains are used to determine whether or not they will neutralize the newly isolated one

The test has been in use for many years both diagnostically and for fundamental research purposes with extremely good results but in no case is it an easy one many animals or chick embryos must be used, and the answer is usually delayed for some time The basic principle of it, as its name implies, is the mixture of a known virus and a serum to be tested or of a known antiserum and an unknown virus and their subsequent inoculation into a susceptible animal or chick embryo Due to the fact that it is relatively expensive and long drawn out it is now little used in the actual diagnosis of infection at least in those cases in which other tests have been devised for the purpose As a research procedure, on the other hand it is of the greatest value and is widely used

Complement Fixation Tests

These are at present the most popular method of serological diagnosis of virus diseases Inevitably, they depend on the preparation of suitable antigen suspensions, and this in its turn depends on the isolation of the virus in question No specific serological tests are available for the diagnosis of infection unless the causative virus has itself been isolated Even then some time has usually elapsed before a sufficiently potent antigen for these tests has been described At present, complement fixation tests have been described for many of the virus diseases, and they are in routine use for the diagnosis

of mumps, influenza, psittacosis, Q fever, variola and vaccinia, lymphogranuloma and the viral encephalitides

In some cases, e g variola, the procedure may be reversed and the test carried out with an unknown antigen and known antisera Crusts or vesicle fluid, depending on the stage of the disease, are sent to the laboratory for examination, and they are used with known antivaccinial antisera as antigens in the test Similarly, before hæmagglutination by mumps virus had been described, it was common to use the chick embryo tissues or the monkey parotid, whichever had been inoculated, as antigens in the test to see if infection had occurred

The general principles of technique remain the same as in bacteriological practice, with the distinction that in virus work the tests are perhaps more difficult to perform Sufficient controls must be set up with every test It is always possible that the serum under test may show non specific fixation with the tissue moiety of the antigen, and this would not be revealed unless sufficient antigen controls were included in the test All the controls must be carefully considered It was for this reason of inadequate control that so many contradictory reports were published in the early days, when the complement fixation test was first applied to virus work

Agglutination and Precipitation

These tests are now little used in diagnostic work For agglutination tests elementary body suspensions are required, and these have been prepared in sufficient purity only in the case of the larger viruses such as vaccinia and psittacosis They have played a prominent part in the study of rickettsial infections, in which neutralization tests are not so reliable as in the case of the viruses Various tests have been described for the diagnosis of variola vaccinia, but the complement fixation test is now more widely used for this purpose

Precipitins have not been studied very closely, and except for their use in the investigation of the antigens of variola and vaccinia they play little part either in laboratory diagnosis or in research procedures

Hæmagglutination

Certain viruses notably those of the influenza group and mumps have the capacity of agglutinating the erythrocytes of several species including human group O cells In other cases such as vaccinia erythrocytes may be agglutinated by soluble substances associated with the virus particle but not by the particles themselves

Amongst the types of erythrocytes agglutinated by influenza viruses are those of the human ferret guinea pig and chick The reaction obviously represents a very simple method of studying *in vitro* the relationship between the virus and the cell and for this reason a tremendous volume of work has been carried out on it in recent years It is enzymatic in nature and many substances which interfere with it besides specific antibody have been described As will be seen in the chapter dealing with influenza specific antibodies interfere with the reaction and so the test can be used as an index of infection As the agglutination is caused by the virus particle itself the test is strain specific and under ideal circumstances which are not always fulfilled will indicate not only the type of virus present viz A or B but also the strain e g PR8 or A prime

The significance of the research on those substances which prevent agglutination and therefore the union between influenza virus and receptors on the cell (which is probably the starting point of infection *in vivo*) is obvious It is directed towards preventing a similar union between the virus and susceptible cells in the respiratory epithelium Unfortunately although this can be done with experimental mice and infection prevented for a time there is such a rapid regeneration of the receptors on the surface of the cells that these experiments have not been very successful Nevertheless it is possible that by the study of these phenomena a way may ultimately be found to prevent infection at the cell surface

Mumps virus also agglutinates chick cells in the same manner as does influenza but agglutinates human O cells to a much lower titre Hæmagglutination inhibition may also

widely used is the Frei test in lymphogranuloma They have also been used in herpes and in mumps In herpes a good correlation was observed between the skin test using heated amniotic or allantoic fluid antigens and the serum antibodies In mumps the *reaction may be elicited either with monkey* parotid gland or with a yolk sac antigen There is no correlation with complement fixing antibodies, but the incidence of subsequent attacks of mumps is largely confined to those with negative reactions

CHICK EMBRYO TECHNIQUES

The introduction of the fertile hen's egg as a medium of growth for viruses marks the biggest single advance in the history of virus study It had been known earlier, but it first began to come into routine use after Woodruff and Goodpasture had used it in 1931 for the growth of fowl pox virus Since then it has been employed on an increasing scale, and techniques have been described for the cultivation of most of those viruses with which we are acquainted in one or other of the cavities of the embryo If any one name can be associated with these advances it is that of Burnet Most workers modify his techniques in details but almost all of them follow the broad lines of his descriptions A most valuable monograph by Beveridge and Burnet, published in 1946, has collected all the available technical data on the management and inoculation of fertile eggs, the following account is based largely on the methods given therein

Some of the many advantages possessed by chick embryos over other methods may be emphasized They reduce very materially the need for large stocks of laboratory animals, without which no work could otherwise be carried on There is very little danger of cross infection with proper techniques Most important of all they are not, so far as we know, infected with any viruses themselves, and so none of the latent viruses which may confuse the results in experimental animals need

are a reasonable degree of manual dexterity and the main tenance of bacterial sterility This latter is necessary becaus bacteria, once introduced into the egg in any number, wi grow profusely and will kill the embryo Fortunately th sulphonamides, penicillin and streptomycin are powerfu weapons at the disposal of the virologist in the laboratory Without any effect on viruses themselves they will if inoc lated with the virus, kill off bacterial contaminants This i of particular importance in the primary isolation of viru from, e g throat washings or crusts which are almost certai to be contaminated by many organisms

Anatomy of the Chick Embryo

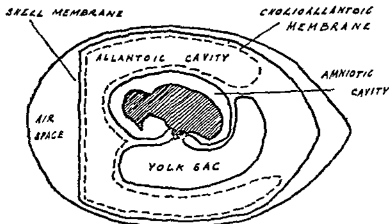


FIG 1 Semi diagrammatic sketch of chick embryo to show the relative positions of the cavities mentioned in the text

Little is to be gained by giving a detailed description of the development of the chick embryo for which the larger works, or the monograph by Beveridge and Burnet, should be consulted Some idea of the various cavities which are used by virus workers is however necessary For this purpose, an embryo of twelve to fifteen days incubation, when these

structures are fully formed is described On reference to Fig 1 a clear idea of the broad outline of the anatomy of the embryo can be obtained

If one assumes the embryo itself to be situated in the centre of the egg the various cavities and membranes will be described with reference to it as a central point It is almost completely surrounded by the *amnion* and lies bathed in *amniotic fluid* which measures up to 1 ml in amount Any material inoculated into this cavity is in contact with the cells of the embryo will be swallowed and will also enter the respiratory tract

Connected with the abdominal cavity of the embryo is the *yolk sac* which in the first few days of incubation occupies the greater part of the egg As the embryo draws on the yolk for its growth the sac becomes progressively smaller and it disappears completely before the chick hatches out

The *allantoic cavity* which occupies most of the remainder of the egg is a relatively large space containing between 5 and 10 ml of fluid It is particularly used for the production of large amounts of virus for experimental work

Bounding the allantoic cavity is the *chorio-allantoic membrane* a thin very vascular structure which is the respiratory organ of the embryo On its outer aspect this is in close contact with the shell membrane which in its turn lines the shell and at the wide end of the egg forms the air space

Inoculation Procedures

The eggs are incubated until required in an ordinary chick incubator at 38–39 C and are usually turned and cooled for ten minutes twice daily during this period They should not be kept more than ten days from the time they are laid before being incubated The length of incubation before inoculation depends on the techniques to be used For inoculation into the yolk sac eggs are used at five to six days into the allantoic cavity at ten to eleven days on the chorio allantoic membrane at twelve days and into the amniotic cavity at thirteen to fourteen days For certain special purposes these times are varied

Before use the eggs are *candled* to see if development has taken place This consists in transilluminating them, preferably in a completely dark room, and in a fertile egg the dense shadow of the freely moving embryo can be seen Other landmarks, such as the allantoic vein and the vessels of the chorio allantoic membrane, can also be made out

Drilling

Openings in the shell are usually made with a small electrically driven drill A portable dental drill with a carborundum disc is very satisfactory Each laboratory will have its own particular machine, but the general principles of all are the same As is described later the drill is used only for opening the shell and the shell membrane must not be damaged in the process With a little practice the technique can easily be mastered

Yolk Sac Inoculation

The technique of yolk sac inoculation is simple After five to six days incubation the sac occupies most of the area of the egg *A small hole is drilled in the wide end and the inoculum injected blindly with a syringe and needle* The needle is passed through the hole in the shell and the air space until it reaches the centre of the egg in its long axis 0 25 ml is the usual quantity injected In yolk sac work it is very important that absolute bacterial sterility be achieved, as the yolk sac represents an ideal medium for the growth of bacteria

This route is used principally for the growth of rickettsiæ and the psittacosis lymphogranuloma group

The hole in the shell is sealed with a mixture of melted vaseline and paraffin wax, and the eggs are placed upright in an ordinary bacteriological incubator at 35°C They are candled twice daily to see if death of the embryo has occurred and are harvested as soon as possible after death The yolk sac is removed and smears made from it *These are stained* by Castaneda s or any one of the other similar methods which will demonstrate the elementary bodies or the rickettsiæ

Allantoic Inoculation

Inoculation into the allantoic cavity is even more simple Ten or eleven day eggs are candled and an area chosen where the membrane is well developed, but where there is a clear space between the blood vessels A small hole is drilled down to, but not damaging, the shell membrane and the injection made blindly The needle is kept as close to the horizontal as possible during the inoculation The hole is sealed and the embryos returned to a bacteriological incubator, where they are kept upright with the wide end upwards

Owing to the large yield of infected fluid obtained this method is principally used for the routine passage of strains of influenza or for the production of influenza virus vaccine It is not by any means as sensitive as the amniotic route for the primary isolation of this virus

Eggs inoculated allantoically with influenza are harvested after about forty two hours incubation After removal from the incubator they are chilled for not less than two hours in the refrigerator This ensures that little bleeding from the torn vessels of the chorio allantois will be encountered during harvesting Should bleeding occur the virus is adsorbed on to the embryonic erythrocytes with a consequent drop in titre In some cases advantage is deliberately taken of this adsorption and bleeding is encouraged, so as to obtain a concentrated suspension of the virus After its adsorption on to the cells the virus is then allowed to elute into a small volume of saline—less than the volume of the original fluid

After chilling the egg is put standing upright and the shell over the air space is chipped away The shell membrane and the chorio allantois lining the bottom of the air space are then cut or torn across and the fluid aspirated with a Pasteur pipette The presence of infection with influenza virus is then confirmed by hæmagglutination tests

Chorio allantoic Membrane Inoculation

This route is used principally for the pox viruses and those others which produce specific lesions on the membrane

Twelve day eggs are candled the air sac is outlined and a well developed area of the chorio allantoic membrane selected Over this a triangle is outlined with its sides some 12 mm in length After sterilization with alcohol the shell is drilled along the sides of the triangle down to the membrane In addition a small opening is made into the air space

The triangle of shell is then gently raised with a mounting needle or vaccination lancet and is discarded A drop of saline is then placed on the exposed shell membrane With great care not to damage the underlying chorio allantois for damage will produce subsequent non specific lesions a small nick is made in the shell membrane Suction is then applied with a rubber teat to the hole in the air space the saline runs in between the shell membrane and the chorio allantois and the latter will be seen to fall away Thus an artificial air space is created under the window in the shell The triangular area of shell membrane is then removed and the inoculum dropped on to the membrane

The window must then be sealed This can be done with a piece of scotch tape but it is not always easy to be sure that the egg is completely sealed with this and a better method is to use an ordinary microscopic coverslip The vaseline paraffin wax mixture is melted and with the aid of a Pasteur pipette a parapet is built up around the opening A flamed coverslip is then placed on top of it so that an airtight seal is made

During the subsequent incubation period of two to three days the egg should not be disturbed At the end of this time it is opened placed on a piece of cotton wool impregnated with antiseptic and the sealing material removed The shell is flamed and then cut or broken away so as to allow the fullest exposure of the chorio allantois This is cut around the periphery and removed to a Petri dish containing saline to be examined for specific lesions

Amniotic Inoculation

The technique of amniotic inoculation requires rather more

skill than the other methods if good results are to be obtained. Unless care is taken the inoculum will not be placed in the amniotic cavity As already mentioned, any material inoculated here is in direct contact with the cells of the embryo and the method is potentially a most valuable one In practice, however, its main value to date has been in the primary isolation of influenza virus from throat washings For this purpose it is probably the most sensitive method at present available

Eggs of thirteen to fourteen days incubation are used after candling to determine the position of the embryo This may be done by direct inspection and choosing the area of greatest density which in practice is not always easy to discover Another method is to candle the egg with the wide end towards the operator and to note the position of the allantoic vein where it arises close to the air sac The egg is then rotated in a clockwise direction for about half an inch, and a point marked on its greatest diameter The embryo lies under this spot

Irrespective of which method is used the preliminaries are the same as for chorio allantoic inoculation A triangle is drilled in the shell over the area selected the shell removed and the chorio allantois dropped by suction over the air space In practice this latter technique may not be necessary, as it will be found that the drop of saline can be persuaded to run in once the shell membrane has been nicked, and the membrane will fall away without suction When the shell membrane has been cut away the embryo should be seen through the chorio allantois directly underneath the opening A small hole is made in an avascular area of the membrane and the amnion gently drawn up through the opening The inoculation is made with a fine pointed Pasteur pipette into which a little air has been drawn up after the inoculum, at the base of the tag of amnion which has been pulled up With the pipette in position the air is injected first If the tip of the pipette is in the amniotic cavity the bubble of air will remain clearly visible and the rest of the inoculum can follow

If the bubble floats away to the side the pipette is in the allantoic cavity, and another attempt must be made to insert it into the amnion

After amniotic inoculation it is best to seal the egg with a coverslip as described above because this facilitates inspection during the subsequent incubation period which is usually four or five days The eggs are examined daily and any embryos dying within the first forty eight hours are discarded Those dying subsequently are harvested It is usually an easy matter to determine the life or death of an embryo through the coverslip as its movements will be clearly visible if it is alive

If the egg has been inoculated for the isolation of influenza the amniotic fluid is harvested with a fine pointed Pasteur pipette and tested for hæmagglutinins It is diluted 1 10 for the test because non specific hæmagglutination may occur due to the high albumen content of the fluid If the test is negative the fluid is passed undiluted into a further batch of embryos Should this second passage also prove negative it is fruitless to continue The embryo lungs may also be ground up and used for passage or histological preparations may be made in the ordinary way

For the isolation of mumps virus amniotic inoculation into eight day embryos is used After incubation for four to seven days infection may be recognized by testing the amniotic fluid for hæmagglutinins or by using the embryo as antigen in a complement fixation test with known antisera

TISSUE CULTURE

For diagnostic or routine work tissue cultures have been little used since the introduction of chick embryo methods but they still play an important part in research procedures They are of particular value in the study of the morphology of viruses and many of the life cycles described have been worked out in such cultures Numerous techniques are now available In most of them minced embryonic *tissues* are

suspended in plasma or serum or in a nutrient medium such as Tyrode's solution It is usual to replace the fluid at regular intervals while an experiment is in progress The cellular elements may be ground up and transferred to fresh cultures, or they may be stained and examined microscopically Recently it has been reported that the Lansing strain of poliomyelitis has been grown in tissue culture using human tissues

EXPERIMENTAL ANIMALS

Such a wide range of animals may be used for virus work that little is gained by cataloguing them They have the advantage, as already noted, that serological reactions as well as the specific lesions produced by a virus may be studied On the other hand, these animals themselves are subject to virus diseases, which especially when they have a tendency to latency, may readily confuse the experimental results obtained With increasing experience these disadvantages may become of less significance, and animals remain essential for the study of viruses

Chapter Three

THE CONTROL OF VIRUS INFECTIONS

This is a *chemotherapeutic age and we have now reached* the stage when it can be said that there is an excellent prospect of some measure of control over the bacterial infections Whether we shall ever attain complete success is doubtful The balance between man and the pathogenic micro organisms is delicate, and the relatively crude methods of wholesale chemotherapeutic or antibiotic slaughter which are now in vogue seem unlikely to provide a permanent solution *The results have been very good so far, but bacteria* are already developing highly efficient resistance mechanisms, insensitive strains are appearing more frequently, and there is a very real danger that the indiscriminate use of these agents may ultimately render them almost useless In fact, it is fortunate that we no longer rely on penicillin or streptomycin alone for much of their efficacy may have already been squandered

With the question of the bacterial infections on the road to solution practitioners and epidemiologists are now being forced to turn their attention to the virus infections There the immediate prospects are not nearly so bright We are poorly equipped for an encounter with the viruses While there have been tremendous advances in the techniques of virus study during the past decade our present methods of diagnosis of prophylaxis and above all, of treatment are still inadequate

For the prophylaxis of virus diseases we may borrow from previous bacteriological and immunological studies, and the *result has been that the available methods of prophylaxis* are, without being adequate, far more efficient than those of

treatment In some cases we can now prevent an infection which once it occurs, we cannot cure

On the other hand the specific treatment of virus disease is a completely new field We may have some empirical successes, but to put the subject on a scientific basis it will probably be necessary to await further technical advances in the examination of viruses themselves Previously we knew little of the problem and are only gradually beginning to realize how difficult it is but the constant progress both in techniques and in the field may bring a solution nearer than seems to be possible at present

METHODS OF CONTROL

It is necessary to try and correlate the needs of the practitioner who deals only with the patient and his immediate contacts with those of the epidemiologist who sees the patient in the mass Both would be satisfied if infectious disease could be completely prevented When in spite of precautions, infection does occur their goal is still the same even though for different reasons One wishes to cure his patient quickly the other wants the patient cured and rendered non infectious quickly and efficiently so that no widespread outbreak can occur At present and for some years to come the main weapon at our disposal must be prevention—by whatever means attained Here the practitioner the epidemiologist and the laboratory worker must work in conjunction

The means of prevention and control are varied Specific measures such as immunization and better treatment, and the more general such as improved hygiene and better housing, can all play their part They must be used intelligently and in conjunction and even then we cannot tell how they may influence the epidemiology of any particular infection Our very success in improving hygiene and sanitation seems to have raised poliomyelitis from an unimportant endemic disease to a major epidemic one

Three entities must be kept constantly in mind in dealing with virus diseases—the virus, the individual and the community It is only by watching the interplay of these three that any measure of control can be envisaged In the treatment of infectious diseases the emphasis is laid first on the cure of the individual patient, and only later, and almost incidentally, is any attention paid to preventing the spread of infection to others When we come to consider prophylaxis the community is the unit under discussion, and one attempts to keep the whole population free from, or immune to, the disease

This latter may be done in various ways—naturally or by design It may happen naturally from time to time, as shown in any population after an epidemic Most virus diseases are self limiting, the number of cells in the body which can support the growth of an infecting virus is limited The victim of a severe attack either dies rapidly or recovers, in some cases completely, in others with sequelæ which remain for life Convalescent carriers do not seem to be of much importance From the view point of the community this means that the patient does not remain infectious for long and, as most infections caused by viruses confer a solid immunity on those attacked, all those who survive any one epidemic will not have to be protected further This is an expensive method of raising the immunity level of the population and, quite apart from the suffering involved, is not very efficient, because the number of new immunes may be balanced by the number of deaths and the community as a whole is little better off

None the less, without going deeply into epidemiological theories, it is clear that it is important to keep the immunity level of the community, or herd, as high as possible At present it is probably the most important single weapon at our disposal for the control of virus infections In a completely susceptible population measles is a killing disease while smallpox is of little significance in a vaccinated population

It is difficult to keep up this herd immunity deliberately, and we must rely to a very considerable extent on the virus itself doing it for us. For example, if any virus disease is widespread and numerous cases are occurring, we know that the amount of virus in the environment is so great that by either clinical or sub-clinical attacks almost the whole population has the opportunity of becoming immune.

Theoretically it is possible to raise the immunity of a population by artificial means but the labour and expense involved would be enormous. Therefore as a rule we compromise. Certain individuals at special risk, e.g. nurses in a smallpox hospital, are protected. Alternatively certain sections of the community are singled out as are infants in those countries in which vaccination is compulsory. Only in the face of an epidemic of smallpox does one mass vaccinate and try to protect everyone. Even then such measures are combined with general hygienic ones such as isolation and quarantine.

This is reducing the problem to its simplest terms and is in fact an over-simplification for one cannot prophesy with confidence how any virus will behave and it is almost impossible for the epidemiologist to estimate the level of the herd immunity. Even if a population appeared to be almost completely immune due to a recent prevalence of virus we still could not afford to relax. Most would have thought at the end of 1947 that the population of England was secure from a large poliomyelitis epidemic for some time to come. Yet within two years another epidemic was in full swing which was followed by a third within the succeeding twelve months.

The ultimate aim of prevention by whatever means it be achieved is to deny the virus access to susceptible cells. With the possible exception of measles, which can now by sero-attenuation be converted into a mild infection with a subsequent lasting immunity, it is obviously better to prevent any infectious disease occurring at all than to try and cure it later. The subject may be discussed under several headings—better

hygiene, earlier diagnosis, better treatment and isolation of cases as they arise, and prophylactic inoculation The main emphasis will here be placed on the last group, but it must be understood that all the methods go hand in hand It is frequently simpler and more effective to eradicate a disease by, for instance, destroying insect vectors than it is to immunize a whole population against it Yellow fever is an excellent example of this approach Again, with smallpox it is obviously better to have proper diagnostic and isolation facilities available to deal with an outbreak, than it is to mass vaccinate when the epidemic is already in progress and out of control

PRINCIPLES OF THERAPY

The whole emphasis in the modern treatment of infectious diseases is directed towards a specific attack on the micro organisms responsible This trend, which may be said to have begun with Ehrlich, has been intensified in our own era Our methods are yearly becoming more precise due to improved techniques which enable us to study the bacterium, its likes and its dislikes, and to work back from there As a result of greater knowledge the metabolism of the bacterium is studied and we find substances with which to feed it, substances which will either kill it directly or render it more amenable to attack by the natural defence mechanisms of the body The empiricism of the past is being replaced by a planned approach

In transferring this method to the virus field we *immediately* run into a very real difficulty, and one which is inherent in the nature of viruses themselves That is their intracellular habitat Viruses are so parasitic as to have little or no metabolic activity of their own, and they rely on the cell to provide it for them The virus grows in the cell, at the expense of the cell and only when the cell is destroyed and fresh virus particles are liberated to attack others does it appear in the open

One cannot at present outline any broad principles for the treatment of virus diseases Each must be considered separately, not only in respect of the methods used but also in respect of the criteria of cure The aim of treatment in any disease is the cure of the patient and his return to normal health In infectious diseases it is also necessary to ensure that the organism causing the disease has been completely eliminated Even in some bacterial diseases, e g typhoid, this is not always achieved and the patient, although clinically well, may become a carrier in whom the typhoid bacilli persist long after apparent cure

Something analogous to this may also happen in some of those virus diseases in which successful chemotherapy has been claimed We know, for instance, that the virus of herpes simplex remains in the tissues of many cases after the primary infection, without ever again giving rise to symptoms From some of these cases the virus can actually be isolated Psittacosis, one of the large viruses, is another infection in which a patient has been found actively excreting virus in the sputum as long as eight years after clinical cure It is clear, then, that at least in some virus diseases it may be difficult to speak of a complete cure Clinically the patient is perfectly well but the virus still persists in his tissues

In other cases, for example yellow fever, the end results seem to be better in that we have not been able to demonstrate the virus after "cure" Even here some workers are not satisfied but, pointing to the solid and almost spectacular immunity which follows infection with this virus suggest that it may persist indefinitely in the body

There are two schools of thought on this problem One holds that the virus is completely eliminated after "cure" in the great majority of cases The other suggests that after the primary attack the virus does really persist for life, and thereby keeps up the high titre of antibodies which can be found in the blood In this view the regular breakdown of small numbers of infected cells acts as a constant stimulus to the antibody forming mechanism, keeping it at a high pitch

of activity and keeping the circulating humoral antibodies at a high level They say that our present techniques are not capable of demonstrating such a virus

There are obviously two possible reasons for failure to demonstrate such a virus One is that our techniques are inadequate The second is that it is not there Opponents of the theory of continuing infection point out that, except in certain cases such as herpes and psittacosis, it is based on the finding of a high level of circulating antibody for a considerable time after cure In their view it is not necessary to postulate that the virus persists in order to explain the immunological findings One experience of an antigen, which in the present context means one attack by a virus, is sufficient to keep antibody production at a high pitch for years

That such a controversy should take place at all demonstrates the need for a broad outlook in our dealings with the viruses It shows also how little we know of them and of their activities, and how difficult it is to lay down any general principles which would be widely accepted

METHODS OF TREATMENT

Symptomatic Treatment

In the great majority of virus infections symptomatic or supportive treatment is all that we can at present give the patient Our aim is twofold—to help the body defences to prevent further spread of the virus, and to minimize the effects of the damage already done In some instances we are still not clear why certain measures used are of benefit In others we are better off because we now have a reasonably clear idea of the pathology and the pathogenesis of the disease

In bulbar poliomyelitis, for example, we can in the last analysis do very little to help the patient We attempt to prevent death from anoxia in the acute phase and, where the cardiac or respiratory centres are not already involved, to

minimize the strain on them The paralytic prognosis is good if the patient can be brought through the acute phase, but our efforts at this in spite of great advances in recent years are probably not of much assistance In spinal poliomyelitis we use our knowledge of the pathogenesis and pathology of the disease The virus is widespread throughout the central nervous system and strict rest is therefore enforced in the acute stage on those muscles whose innervation is not already clinically affected By this means we avoid insult to those nerve cells and so limit the spread of the paralysis

In measles and smallpox the best we can do is to prevent secondary infection with its sequelæ and dangers By doing so in the former we materially improve the prognosis The effect is less in smallpox but it may make the difference between death and survival

The treatment of yellow fever is entirely symptomatic and is directed towards helping the damaged liver during the acute phase

In none of these diseases therefore is there any question of a direct attack on the causative virus That is left entirely to the body defences which must except in the case of attenuated measles work entirely on their own and with very little support Insignificant though this support may be it is undoubtedly of value and must always be given

Specific Treatment

We now turn to a consideration of chemotherapy in virus diseases Here we attempt to apply in another field those findings of bacterial metabolism which are the basis of chemotherapy We cannot use virus studies because the virus cannot be dissociated from the cell in which it lives and we cannot study the metabolism of viruses with any degree of accuracy

Chemotherapy to be of value must be capable of interfering in some way with the metabolism of the infecting micro-organism The question is whether this can be done with

viruses, which have little or no independent metabolism, without interfering with the growth of the cell to as great an extent as does the virus Furthermore, even assuming that an intracellular chemotherapeutic action were possible with any of the substances at present at our disposal, there would still remain the difficulty of getting the substance, fully active, into the cell Probably to deal with a completely parasitic micro organism such as a virus, it would have to be built into the metabolism of the cell itself

In any attempt to attack viruses while outside the cell we must again take into consideration their negligible metabolic activity If anything, the problem is even more difficult than the attempt to attack them inside the cell So far as we know at present the only extracellular activity which could be ascribed to most of the viruses is the effort to enter a cell in order to multiply, and it is doubtful how far this process can be described as active " There is very probably some kind of enzymatic union between the virus and specific receptors on the cell surface before the virus actually enters the cell Numerous experiments have been carried out with influenza virus and a receptor destroying enzyme present in filtrates of the cholera vibrio Unfortunately, while the receptors can be inactivated for a time, they regenerate rapidly, and the method cannot be regarded as more than a starting point for future research

Any positive result in the chemotherapy of virus diseases is of great interest Apart from the value of treatment it increases our knowledge of the biological activities of the viruses affected If a virus were amenable to attack by any of the products in use today, it must be done, so far as we know, *outside* the cell From this it follows that the virus in question would have some activity, *however slight*, outside parasitized cells, other than its preliminary union with the cell surface

There is no question but that chemotherapy is now effective *against rickettsiæ, and it is significant that the only viruses* for which successful results have been consistently claimed

are those of the psittacosis lymphogranuloma group The biological position of this group is still obscure, and at present it is probably best to consider it as midway between the viruses and the rickettsiæ

Even with these viruses it is difficult to know where we stand, because their capacity to persist in a latent form after clinical cure must be taken into consideration One obvious advantage of chemotherapy in these cases is the elimination of secondary bacterial invaders It may well be that in this group the virus action of itself, once the acute stage is passed, could be so mild as to pass clinically unnoticed, and in assessing the results it may be necessary to make a distinction between clinical cure and destruction of the virus In trachoma for instance it is often still possible to demonstrate inclusion bodies after adequate dosage with sulphonamides has resulted in apparent clinical cure The results with penicillin are similar

No successful chemotherapy has yet been claimed with any confidence for those viruses whose position is less ambiguous, for example poliomyelitis, yellow fever, or smallpox

To summarize the present status of treatment, it will be seen that the methods available to us remain empirical in most cases Chemotherapy and the antibiotics have been disappointing Their very success in dealing with bacteria has exposed them to use in virus infections for which they were never intended The only virus diseases in which any good effect by them has been claimed are those of the psittacosis lymphogranuloma group whose biological position is not yet finally settled Even here there is controversy as to their real value

The newer antibiotics have so far added only one disease to the small list—primary atypical pneumonia Good results are also being claimed in the treatment of such infections as herpes zoster hepatitis and varicella but these claims still require more critical evaluation In the case of zoster, for instance the condition has actually appeared while treatment with aureomycin was being carried out for another conditio

PROPHYLAXIS

From what has already been said it is clear that not only are our methods of treatment not very good, but also that we can prevent the occurrence of some infections which, once established we cannot cure It may be recalled that smallpox is one such condition, that it is liable to become epidemic in any western European country at any moment, and yet that compulsory vaccination has been abandoned in many of them In view of this it may fairly be stated that the outstanding success of preventive medicine in the virus field during this century has been in the case of yellow fever By means of yellow fever vaccine and anti mosquito measures—*an example of inoculation and hygienic measures being* supplementary—the economic future of South America and Central Africa has been made perceptibly brighter Perhaps *more important, it is now possible to undertake* laboratory research on yellow fever without the appalling risks to personnel which previously obtained

It should be admitted at once that the position is not nearly so satisfactory in the case of the other virus infections True, measles can be prevented altogether or attenuated by the use of gamma globulin but mumps, chickenpox and rubella still elude such methods of control Above all poliomyelitis remains an enigma Not only is no form of specific treatment available for it, but no specific prophylaxis has yet been described

Influenza is in a category by itself Protection can be afforded by vaccination with inactivated virus but such an immunity is of short duration Further, the timing of the injections and the choice of the strains of virus to be used in the vaccines are matters of great importance If the inoculations are given too long before an epidemic the immunity produced may have waned before the outbreak occurs Conversely, if they are delayed too long antibodies may not gave time to develop before the patient is attacked There must also be a close antigenic relationship between the

strain of influenza virus in the vaccine and that causing the epidemic, or no protection will be elicited

On general bacteriological principles it is to be expected that in dealing with viruses active immunization should be more successful than passive In fact the gap between them is even wider than is the case with bacteria The intracellular location of virus activity is an obvious reason for this The aim of prevention has already been discussed—to prevent the entry of virus into the cell Active immunization, by the antibodies it elicits, can play a part in this for a considerable period after the injection Passive immunization is of such short duration that the timing of the injection is critical

The pathogenesis of the exanthemata as postulated by Fenner may be recalled, for it has a direct bearing on the value not only of immunization but also on the prospects for specific therapy One should be cautious about applying his findings in inoculated mice to human infections but they do provide a working hypothesis

On an analogy with his work on mouse pox it seems that after infection and during the incubation period the virus proliferates in some internal organ such as the spleen These infected cells break down leading to a viremia, which infects the skin and gives rise to the specific rash When gamma globulin which is a form of passive immunization is given early in the incubation period it is capable of modifying the stage of viremia and preventing the subsequent skin localization of the virus Should the gamma globulin be given too late to modify the viremia then obviously no modification of the attack would take place

If this be the pathogenesis of the exanthemata it would seem that once the stage of viremia has occurred it would be already too late for either immunization or chemotherapy. In other words in the case of virus diseases we may have to ct immediately after exposure to infection and long before nical signs appear To wait until they do means dealing th the infection at its peak and not as we have been ustomed to think at an early stage

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This explanation obviously does not cover all virus diseases and would apply only to the exanthemata Influenza, with its short incubation period and superficial location of the cells attacked, is an outstanding exception At no stage is there a viræmia in influenza What is needed here and what apparently occurs after active immunization is a local concentration of antibody at the place where it is most needed—the respiratory tract Passive immunization is of no value

The value of active immunization is associated with the whole problem of immunity in virus diseases Many questions arise here which do not, on the whole, trouble us in dealing with bacterial infections As a general rule immunity after a virus infection is solid and lasts for life In some cases it is spectacular, as in the case of yellow fever where circulating antibodies have been found seventy five years after an attack In addition serological surveys have shown numerous instances of immunity to various infections, as judged by the detection of humoral antibodies, in persons who give no history of having suffered a clinical attack of the disease It would seem therefore that immunity can follow a sub clinical or latent infection as readily as it can a frank attack Furthermore, such infections must be very frequent

Such findings based on humoral antibody surveys, have led many workers to question the significance of circulating antibodies in virus infections and also, as has been seen to question whether or not recovery from infection with a virus is ever as complete as it appears to be

As to the significance of circulating antibodies it is relatively common in some cases to isolate virus from the immune subject, e g in the case of lymphocytic choriomeningitis of mice With this in mind many consider that such antibodies are of little value in prophylaxis, and that only a tissue immunity, based on an immune cell which has been altered in some way to make it refractory to infection, is of any significance This is an extreme view and seems to be running well ahead of the facts at our disposal at the present time Against it there is ample evidence to suggest that circulating

cases in which the virus has been grown technical difficulties in the preparation of the vaccines are all that stand between us and success

It is necessary, of course, to preserve a sense of proportion in these matters In a number of those infections in which there is marked danger to the life or the subsequent well being of the patient rapid strides have been made There is a danger that the spectacular advances recently made in the control of the bacterial infections may unfairly overshadow the success of virus workers, who are in a much more difficult field Our very success with bacteria has brought us up sharply against the virus problem and by comparison it may at times seem insoluble That it is far from being so is shown by the advances made during the past decade It is necessary, however, that physicians, epidemiologists and laboratory workers should work in conjunction, and that the information derived by one group be made fully available to the other

There will unquestionably be setbacks for we are dealing with micro organisms which have an almost infinite capacity to appear in new variants Our very success in producing variants in the laboratory, such as the attenuated strains used for active immunization, shows that the same process occurs in nature even if more slowly The apparent disappearance of the PR8 strains of influenza and their replacement by A prime strains is an excellent example of such a change Another, and more spectacular, example was the appearance of the virulent pandemic influenza strain in 1918–19 Nevertheless we are slowly coming to grips with the problem Our first duty is not so much to solve it as to isolate it Not until we know how a thing happens can we do much to prevent it

work at the time Swine influenza first noted in the United States during the last pandemic, is now known to be due to the synergism of a virus, closely related to the human types and a bacterium (*H suis*) of the same group as Pfeiffer s bacillus However, we are still ignorant as to whether some such synergism occurred in 1918–19, or whether an abnor mally virulent strain of influenza virus was responsible Neither theory is likely to assist in forestalling another pandemic

EPIDEMIC INFLUENZA

The scientific history of influenza goes back only to 1933 when Smith, Andrewes and Laidlaw first transmitted the human disease to ferrets These developed an influenza like illness and showed neutralizing antibodies during convales cence Great as was this advance it soon became clear that the virus described by these workers did not account for all cases and it was not until 1940 when Francis isolated another virus, closely similar to the first but antigenically quite distinct, that the cause of influenza was finally determined The virus described by Smith and his colleagues was then termed influenza virus A, and that of Francis influenza virus B With the exception of swine influenza virus no others had since been isolated until recently All major epidemics of recent years could be ascribed to strains of A or B, and the significance of C remains to be determined

Clinical Features

Apart from its characteristically sudden onset there is little clinically to distinguish the sporadic case of influenza from a number of other acute infections of the upper respiratory tract Even at the height of an epidemic the diagnosis is more frequently made on epidemiological than on clinical grounds The site of action of the virus—the respiratory epithelium—and the consequent high concentration of virus in droplets, together with the short incubation period readily

explain the rapid ...
It seems likely th...
in two stages—fi...
on the cell surface, and then its entry into, ... specific receptors
the cell Once a cell is destroyed a vastly increased number of virus particles are freed to repeat the cycle and a clinical attack occurs when a sufficient number of cells has been destroyed

In a small proportion of cases pneumonic complications occur which are usually due to secondary bacterial invasion but the virus itself may also occasionally cause pneumonia The number of such complicated cases is small but the mortality from influenzal pneumonia when it supervenes, is high

The pathological lesions associated with influenza vary, according to the severity of the case, from pharyngitis and tracheitis to bronchiolitis and bronchopneumonia

Epidemiology

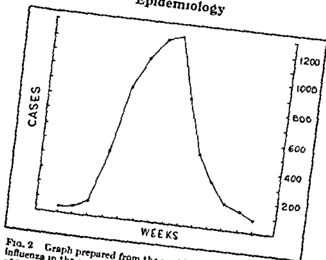


FIG. 2 Graph prepared from the weekly returns of deaths from influenza in the great towns of England and Wales in the winter of 1950-51 It demonstrates the rapid development and decline of an influenza epidemic

The disease is world wide in its distribution, epidemic which build up rapidly to a peak and as rapidly die down occurring during the winter months

The virus is present in the upper respiratory tract during the acute phase and is spread by infected droplets, a case being infectious for a short while before and two or three days after the onset of symptoms Healthy carriers have been blamed for outbreaks in remote communities by introducing infection, but while virus has been isolated from apparently healthy persons during an epidemic, the existence of carriers has not yet been proved The problem is bound up with the unknown reservoir of the virus between epidemics It has been suggested that after an epidemic an altered or degraded form of the virus persists, which becomes virulent again when conditions are favourable Alternatively, influenza may persist by means of small outbreaks and isolated case to case *transfer in non-epidemic periods A third suggestion taking* into account the close relationship between the viruses of human and swine influenza, postulates the hiding place of the human type to be in the pig population None of these theories can be taken as proven, but at the moment continued case transfer appears the most likely

Epidemics of influenza occur every few years with a probable two to three year cycle for influenza A, and four to six years for B, which seems to be more frequently concerned with sporadic cases These cycles fit in reasonably well with observations since 1933, that is since we have been able to tell the ætiology of any outbreak with certainty, although on at least one occasion an expected epidemic failed to materialize It seems most likely that the governing factor is the balance between susceptibles and *immunes in any* population in spite of the remarkable and disconcerting power of the virus to appear in almost completely new antigenic forms, as happened in 1947 For about a year following an epidemic the immunity level of a community remains high to the strain which caused it, but other factors of which we are still ignorant play an important, if not a decisive, part

Even a susceptible person will not develop influenza unless he, or she, comes into contact with the virus Only when we find its hiding place between epidemics can we place the epidemiology of influenza on a sound basis

Properties of the Virus

On electron microscopy influenza viruses show as round or ovoid elementary bodies about 80 to 100 $m\mu$ in diameter according to the type Recently filamentous forms up to 1μ in length and 100 $m\mu$ across have been described in freshly isolated strains, these appear to be stages in the life cycle of the virus

The elementary bodies are infective, agglutinate the red cells of various species and give rise to antibodies on inoculation Also contained in them, but separable from them by the high speed centrifugation of infected fluids, is another important antigenic constituent, the so called 'soluble substance" This consists of smaller particles, about 10 $m\mu$ in diameter, which are non infectious, non agglutinating and non immunizing Its importance lies in the fact that while both fractions may be used as antigens in the complement fixation test for the diagnosis of influenza, the soluble substance appears to be more efficient

The types of influenza virus A and B are antigenically quite distinct, but within each of them there may be marked strain differences Type variance is due to qualitative differences between antigens while strain variance is apparently due to quantitative differences amongst the same antigens When elementary body suspensions are used either for experimental or diagnostic purposes differences between strains are of very great importance The soluble substance of all strains of the same type is, however identical Hence a complement fixation test using the soluble substance as antigen will distinguish between infection with A or B virus, for example, but will give no information as to the identity of the infecting strain, or its relationship to other strains of the same type

One of the most interesting properties of influenza virus is the capacity of the elementary bodies to agglutinate the red cells of various species, including amongst others, man, chick and guinea pig The reaction depends on an enzymatic reaction between the elementary bodies and specific receptors probably mucopolysaccharides, on the cell surface After the virus has been adsorbed on to and has agglutinated the erythrocytes, it may be removed or eluted from them at 37°C Killed virus, while it agglutinates, is not capable of elution It is obvious that this phenomenon—called the chick cell agglutination reaction (CCA)—is a rapid and simple method of testing for the presence of virus in infected fluids and of titrating it if present Certain other viruses notably mumps and Newcastle disease of fowl, share this property with influenza It is very probable that a similar reaction takes place at the surface of susceptible cells during infection with influenza before the virus enters the cell to multiply There is, of course, no multiplication of the virus while in contact with erythrocytes

The CCA reaction is of value in the titration of infected fluids, but of greater importance is the observation that antibodies to influenza inhibit the reaction, thus providing us with a simple technique for the detection and estimation of these antibodies This property is very widely used in the laboratory diagnosis of influenza as the CCA inhibition or Hirst test

While the natural habitat of influenza virus is the respiratory tract of man it can readily be adapted to certain laboratory animals, notably the chick embryo, ferret, hamster and mouse For diagnostic purposes the most widely used is now the chick embryo Either the allantoic or the amniotic cavity may be used the latter being far more delicate It will support the growth of much smaller quantities of virus in, for example throat washings an important point when one is attempting primary isolation in the face of an epidemic It is considerably easier to isolate the virus during an epidemic than from sporadic cases

... must remember that almost all the work which has been done on influenza virus has been done on laboratory adapted strains, and we know little of the relationship of these strains to the behaviour of the virus in its natural state. The discovery of filamentous forms in recently isolated strains is therefore, a timely reminder of the work yet to be done in this field.

Laboratory Diagnosis

The ideal to be aimed at is the isolation of the infecting strain of virus, but where this is impracticable serological tests are used. With proper techniques these may be relied upon to provide accurate information as to the ætiology of an outbreak.

Isolation of Virus

The virus is abundantly present in the upper respiratory tract during the acute stage and it is then that attempts at isolation are undertaken. Throat washings—or gargles—from the patient are inoculated into susceptible animals, or more usually, into chick embryos in which infection may be recognized by the agglutination of erythrocytes by allantoic or amniotic fluids, depending on the route used. If the latter, histological changes can also be observed in the chick lungs.

The washings must be collected within the first four days of illness and, unless frozen must be inoculated as rapidly as possible after collection. The virus will not survive long at room temperature and washings are usually packed in ice or solid CO_2 (dry ice) during transit. Once in a laboratory they may be stored for months in dry ice at a temperature of $-70°C$ should it not be possible to inoculate immediately.

As far as the practitioner is concerned the essentials are that the washings be taken within the first four days of illness and be packed in dry ice for the journey to the laboratory. Any laboratory dealing with the diagnosis of influenza will provide containers if requested.

Serological Tests

CCA-Inhibition Test

As almost all adults have some antibody to influenza resulting from earlier infection, this test depends on an increase in the titre of influenzal antibody during the course of the illness, there is no titre to which one may point as a diagnostic level Two specimens of blood are required, each taken into a dry tube The first should be obtained within the first four days of illness, and the second a week to ten days later. Both specimens are examined in the laboratory at the same time by an identical technique and the increase in titre, if any, determined Elementary body suspensions, erythrocytes and falling dilutions of serum are employed A four-fold increase in titre is considered diagnostic Since this test is sensitive to strain differences it may be advisable to use several strains of each type, A and B, so as to provide the widest possible cover The value of this was strikingly shown during the European epidemic of 1949 in which the causative strain was only remotely related to strains generally employed If at all possible a strain isolated from the epidemic under investigation should be used

A recent advance of interest has been described by which the CCA inhibition test may be used in the field, giving rapid diagnosis of an outbreak in a matter of hours It is of particular value in those cases in which the outbreak has been in progress for some time before laboratory aid is called for If there are sufficient cases advantage is taken of the fact that both acute and convalescent sera are available and blood is taken from not less than ten acute cases and a similar number of convalescents who have been more than ten days ill These two lots of sera are then examined separately or in pools, using elementary body suspensions of different strains as in the ordinary test, and the antibody titre of each determined *If the geometric mean titre of the convalescents* shows a fourfold or greater increase over the acute phase sera a diagnosis of influenza may be given

COMPLEMENT FIXATION TEST

This test has the advantage that when using the soluble substance only two antigens are required, one to cover A strains, and another for B strains Two specimens of blood may be used, collected at the same time as for the CCA-inhibition test, but if only one specimen is available a convalescent titre of 1 16 is, according to Hoyle, evidence of recent infection The test is technically rather more difficult to perform and is not used so widely as a routine diagnostic procedure.

NEUTRALIZATION TESTS

Once essential for serological diagnosis these tests have now been replaced for diagnostic purposes by the others, although they are still of value for research purposes.

CHAPTER FIVE

MUMPS

(Epidemic Parotitis)

MUMPS as a disease entity has been known from very early times, and accurate descriptions of the parotid swelling are *found in the Hippocratic literature* Two thousand years later little can be added to them As is inevitable in any disease with such a long history a wide variety of causative organisms have been postulated, but the cause is now known to be a virus which has many laboratory characteristics in common *with influenza* virus

Recent advances have led to the recognition of orchitis pancreatitis and meningo encephalitis as manifestations of infection with mumps virus, which may accompany or even replace the parotitis Furthermore studies with the complement fixation test in the United States indicate that *clinically* inapparent infections are relatively frequent The trend of these studies is to indicate that infection with mumps virus does not inevitably lead to parotitis, and that mumps and epidemic parotitis are not necessarily the same thing structures other than the parotid may be *attacked by the* virus

CLINICAL FEATURES

After an incubation time of eighteen to twenty one days there may be a short *prodromal period of some twenty* four hours with pyrexia, headache and sore throat More usually these appear simultaneously with a swelling of the parotid which obliterates the angle of the mandible and is easily seen in front of and below the lobe of the ear Many cases present with pain *in the parotid region as the first symptom* The

skin over the gland may appear red and inflamed but the gland itself is only slightly tender on palpation Nevertheless the swelling gives rise to definite pain and difficulty on

[illegible]

lingual glands on the same side are frequently involved but the latter *may* be *missed* unless *specifically* looked *for* Severe headache lasting for a few days is occasionally present, and if so is a prominent feature

At the beginning the condition is usually unilateral, but within a few days the gland on the other side may have become affected The swollen glands reach their maximum size in forty eight hours, and the swelling persists for about a week to ten days

Constitutional effects are slight and the temperature is rarely high, being dependent on the degree of glandular inflammation In mild cases it has fallen to normal three days after onset Suppuration in the parotids is very rare

A lymphocytosis is found all through the attack and outrules suppurative parotitis It has been reported that the urinary diastase is raised even in cases with no clinical evidence of pancreatitis

Other Manifestations

The involvement of other organs may precede or follow the parotitis, or even occur in its absence For this reason it seems preferable not to refer to such involvement as a "complication"

Orchitis

This occurs in some 20 per cent of cases above the age of puberty, and makes mumps a serious problem if an outbreak occurs in a military camp At the end of the first, or the beginning of the second, week of illness the temperature rises again, and the testis becomes swollen and tender with pain radiating to the lower abdomen and thigh The orchitis,

which is unilateral at first spreads to the other testis in about one third of the cases Recovery follows in a few days *Complete sterility following* orchitis is rare but the majority of cases will show some degree of testicular atrophy

It must be remembered that orchitis may rarely occur as the primary manifestation of mumps

Acute Pancreatitis

This may appear during the second week and presents as a sudden epigastric pain with an accompanying elevation of the temperature This picture is rare and the prognosis is good On the basis of changes in the blood amylase it is possible that there is some degree of pancreatic involvement in every case of mumps It has also been suggested that diabetes may follow an attack but the evidence for this is very tenuous

Ovaritis (Oophoritis)

This is the rarest manifestation of mumps and only a few cases have been reported

Meningo-encephalitis

Clinical evidence of meningo encephalitis is rare, but if the cerebrospinal fluid be examined changes in it will be found in many cases of mumps On lumbar puncture a high percentage of cases show an increase in the pressure of the fluid together with an associated rise in the cell count and the protein The cellular increase is due to lymphocytes

If clinical meningo encephalitis supervenes it comes on four to seven days after the onset of parotitis The patient becomes drowsy with a rise in temperature, headache vomiting and signs of meningeal irritation Orchitis is common in these cases Recovery is the rule and hence little is known of the pathological features of the condition

An occasional case may present with meningitis or encephalitis before swelling of the parotid gives a clue to the

iagnosis In others these may be the only sign of infection, nd unless the virus is recovered from the cerebrospinal uid such cases will not be diagnosed as mumps They seem, owever, to be very uncommon indeed

COMPLICATIONS

These are rare and the prognosis in mumps is excellent, he mortality being very low

TREATMENT

The treatment of an ordinary attack is symptomatic Particular attention must be paid to the mouth which is dry and uncomfortable, lest *suppurative parotitis supervene* No pecial measures are required to deal with the other manifesta ions except for orchitis in which a supporting bandage hould be used In severe examples surgical intervention, uch as decompression, may be undertaken, but the results are not always good and it should be reserved for the occasional very severe case Small doses of stilboestrol have also been used

PATHOLOGY

Singularly little information is available on the pathology of mumps, and nothing of diagnostic value can be derived from histological examination Microscopically the parotid ducts show desquamation of the epithelium and the lumina are filled with necrotic debris There is also infiltration of the interstitial tissues with inflammatory cells No specific inclusion bodies have been described

Only in severe orchitis are any very marked changes found in the testis The whole organ is congested and there are frequent small hæmorrhages The most striking feature is the patchy destruction of the seminiferous tubules

LABORATORY DIAGNOSIS

Within the past few years the techniques of laboratory diagnosis have been considerably improved and several methods are now available In the average case they are hardly needed as the diagnosis is made on clinical grounds but they can be of considerable value in atypical cases As yet the tests are hardly in routine use, but that is principally because they are rarely asked for There is nothing inherently difficult in the tests themselves

Isolation of Virus

Mumps virus is present in the saliva for about three or four days before and after the onset of parotitis It has very occasionally been recovered on the sixth day after onset but specimens should be collected as early as possible Saliva is collected by means of sterile cotton wool pledgets which are placed in the mouth and is expressed from them into an equal quantity of infusion broth It should be gathered over a period of one or two hours

The virus has also been isolated from the cerebrospinal fluid in cases of meningo encephalitis The fluid is collected by lumbar puncture in the ordinary manner

Should there be any delay in their transmission to the laboratory the specimens either of saliva or of cerebrospinal fluid should be frozen in transit They are then inoculated into chick embryos most usually by the amniotic route which is the most delicate but alternatively, the allantoic or yolk sac methods are available Prior to the isolation of mumps virus in the chick embryo the material was inoculated directly into the parotid ducts of monkeys which in positive cases developed pyrexia and swelling of the gland The parotids of these animals may then be used as antigens in the complement fixation test, and complement fixing antibodies are to be found in the blood

Serological Tests

Complement Fixation Test

As in most virus diseases the proof of infection depenc on a rise in titre between a specimen of serum taken early i the attack and another collected a week to ten days late Antibodies to mumps appear about the end of the first wee so that a relatively early diagnosis can be made

Hæmagglutination—Inhibition Test

Mumps virus shares with influenza virus, and other membe of the group, the property of agglutinating the erythrocyt of various species and so a second diagnostic test, hæmag glutination inhibition, is available The inhibiting antibod appears later and remains longer in the blood than does th complement fixing antibody As usual two specimens, on collected early and one late, are needed for the test

EPIDEMIOLOGY

The main incidence of mumps is in winter and spring I occurs most commonly in childhood, but adolescents and youn adults who have escaped as children are often attacke Second attacks are uncommon, one usually giving immunit for life, and the figures quoted for their frequency are ver much exaggerated

Since the introduction of serological tests for infection i has become possible to gain some idea of its real incidence By this means many cases of inapparent infection have bee uncovered in people giving no history of an attack, and ther is now little doubt that mumps is one of the commonest o the infectious diseases

Infection is transmitted from case to case by drople spread Fomites are not of much importance It is probabl that some cases may be infectious and transmit the diseas to contacts in the absence of clinical symptoms How important these cases may be in spreading the disease we d not know Most cases are infectious for about three day

before, and three or four days after, *the onset of symptoms* It is theoretically possible that, in those cases in which the other parotid becomes involved a few days after the first, the virus may re appear in the saliva There is as yet no evidence one way or the other, but it might be of importance in determining the duration of infectivity

Quarantine is usually imposed on contacts for four weeks from exposure, but it is not enforced during the first week As virus appears in the saliva only three to four days at the earliest before symptoms develop, it seems that the latter period might, perhaps, be extended with safety Frank cases are isolated until the swelling has subsided in the affected glands Once again there might be a case for modifying this period in the light of recent advances, which should enable us to demonstrate more accurately the time of the disappear ance of virus from the saliva—whether before, after, or simultaneously with the subsidence of the glands

Control

Convalescent serum, in spite of some favourable reports, would seem to be of limited value, either in preventing infection or in modifying the course of the disease Gamma globulin seems to be little better, but it has been suggested that convalescent gamma globulin might exercise a useful effect, particularly in the prevention of orchitis

Potentially more useful are the attempts to produce active immunity by means of mumps vaccines, but as far as man is concerned this work is still in the experimental stage Inactivated virus stimulates the production of antibodies so *that it is more than likely that vaccines* will eventually be of value Their main use would probably be in the protection of young adults on whom falls the main incidence of orchitis

PROPERTIES OF THE VIRUS

SIZE

By electron microscopy it has been estimated that the elementary body measures 200 $m\mu$ in diameter

SURVIVAL

Heating to 55°C for one hour inactivates the virus but it will survive freezing for some weeks It is resistant to glycerol Other agents such as formalin and ultraviolet light rapidly destroy infectivity

EXPERIMENTAL ANIMALS

Monkeys have been widely used and are easily infected either by parotid or intracerebral inoculation Infection by the latter route produces a meningo encephalitis

CHICK EMBRYOS

The virus grows well in the yolk sac the allantois and the amnion Infection was first proved by using the egg tissues as antigens in the complement fixation tests More recently hæmagglutination tests have been available as erythrocytes of several species are agglutinated by the virus The agglutination is a function of the virus itself and is inhibited by specific antisera As in influenza a soluble complement fixing antigen distinct from the virus particle has been described

No antigenic differences have yet been reported amongst those strains of mumps virus which have been isolated

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CHAPTER SIX

VIRUS PNEUMONIA

IT has been known for some time past that different viruses may play an important part in causing pneumonia but only since the advent of chemotherapy have we been able to assess the problem with any degree of accuracy When speaking of virus pneumonia it is frequently overlooked that several viruses are involved, and too often the terms ' virus pneumonia" and "atypical pneumonia" are equated In fact many bacteria can give a so called "atypical' picture Only recently have pneumonias been considered from the view point of their ætiology rather than of their morbid anatomy The present attitude is a more intelligent one, and more in keeping with a chemotherapeutic age The ways in which any organ can respond to infection are necessarily limited, and similar pathological pictures are often produced in response to different agents

"Virus pneumonia" is therefore a very wide term, and the clinical entity known as *Primary Atypical Pneumonia* forms only a part of the problem In this chapter three types of infectious agent are discussed together because, as will be seen they produce very similar pictures and clinical differentiation between them is almost impossible The properties of psittacosis and ornithosis viruses, and their relationship to the other members of the group of which they form part are more fully discussed in Chapter Seven, but in an attempt to give a coherent picture of virus pneumonia their clinical and epidemiological features are included here Q fever is not a viral infection but its clinical similarity to the other infections and the increasing realization of its wide distribution make it desirable to discuss it in this chapter

PRIMARY ATYPICAL PNEUMONIA

There are many objections to this title, but it has become sanctioned by usage and covers a more or less well defined clinical syndrome, in which cold or streptococcal agglutinins are often found in the patient's serum For the present, therefore, it is better to retain it than to introduce further confusion by inventing other names

During the war a considerable volume of work was presented on this subject, but despite very complete investigations by competent workers little real progress was made, and laboratory interest in the condition has largely lapsed Clinical interest remains unabated, and there is a widespread impression that these cases are becoming more common; certainly, the diagnosis is being made more frequently

There are reports in the literature of several different viruses which have been isolated from patients presenting this clinical picture The only one which has so far been fully investigated and which appears to be of importance, is that described by Eaton and his collaborators in a series of papers from 1942 onwards First isolated from sputum and lung suspensions of cases of atypical pneumonia, it can be transmitted to laboratory animals such as cotton rats, hamsters and chick embryos Antibodies to this virus have been demonstrated in many outbreaks in different parts of the United States, and both cold and streptococcal agglutinins have been present in most of these sera Eaton has estimated that 60 per cent of all cases of virus pneumonia may be due to this agent

None of the other reported agents can be regarded as being of significance and indeed little confirmatory evidence by independent workers has yet been produced for any of them The great difficulty for workers in this field has been that almost all laboratory stock, and many of the other exotic animals which have been tried, are themselves subject to specific pneumonitis or pneumonia viruses These may remain completely latent in their animal hosts unless activated

in some way. One method of activation may be the injection of material from cases of atypical pneumonia or of other respiratory infections

Many of the human viruses which have been reported from time to time are transmissible to only a limited range of animals, and even in them are only mildly infectious The serological evidence for them, for example neutralization tests, cannot be regarded as convincing

To date, apart from Eaton's virus, the only satisfactory positive evidence of the ætiology of primary atypical pneumonia is the experiments of the American Commission on Respiratory Diseases, in which human volunteers were used These leave little doubt that the cause is a virus

In one of their experiments forty two men were inoculated with pooled sputa and throat washings from cases of atypical pneumonia These washings were sprayed into the nose and pharynx of the volunteers

Twelve men received untreated washings, of whom three developed atypical *pneumonia* five a *minor respiratory infection* and four no illness

no illness

Of the ten cases of minor respiratory illness in the first two groups, three were classified as suspected cases of atypical pneumonia The case of illness in the control group was the one man who was known to have broken isolation and to have come in contact with one of the cases of *minor illness* In view of many of the objections to the reputed isolation of different viruses in animals, it is interesting that there was no "lighting up" of a latent agent in any of the volunteers receiving autoclaved material

The minor respiratory *illnesses seemed to* bear some relationship to atypical pneumonia, although there was a different incubation period and no radiological signs were

present Four of the eleven cases of minor illness showed cold agglutinins to a significant titre and another five showed a rise during the course of the infection

These findings may be important in view of the many mild infections of the upper respiratory tract in which neither bacteria nor any known viruses can be incriminated Such cases may be due to a mild attack of an atypical pneumonia virus On the other hand it is impossible to say from the experiments whether more than one infectious agent was present in the inoculum and it might equally well be that the two clinical pictures were due to two different viruses All this however is pure speculation as no laboratory studies on the infectious agent or agents involved in these experiments have yet been reported

CLINICAL FEATURES

The incubation period appears to be in the region of two to three weeks as judged by naturally occurring small epidemics The onset of an attack is gradual the patient often feeling no more than mild upper respiratory symptoms but the onset of pneumonia itself may be abrupt with pyrexia cough and headache Unquestionably a high proportion of cases remain ambulant and do not feel very ill at any stage Physical signs are slight and X rays reveal a larger lung area to be affected than they would suggest The lesions are usually to be found in the lower lobes In about half the cases only one lobe is affected The radiological findings are striking in view of the relative lack of physical signs but they are not specific in any way and are paralleled by several other conditions

The total and differential leucocyte counts are within normal limits The E S R is raised during the febrile period Only the usual mixed respiratory organisms are found in the sputum and in the early stages at least they are relatively scanty Blood cultures remain sterile

The duration of the fever is variable and it may range from a few days to two months but in the average case the

temperature is never very high, and will fall by lysis at the end of ten to fourteen days Bradycardia is frequent

Complications are uncommon and generally insignificant The newer antibiotics such as aureomycin, chloromycetin and terramycin are of considerable value in these cases

The mortality is only about 0 1 per cent so that there are few descriptions of the pathology The morbid anatomical picture is hardly distinctive, consisting of an irregular patchy bronchopneumonia with bronchitis and bronchiolitis

RADIOLOGICAL FINDINGS

The most common appearance is a wedge shaped zone of consolidation in the lower lobes, with the apex towards the hilum and the edge fading out irregularly into the lung substance Occasionally mottled or feathery infiltrations are seen Dense opacities are rare

Diagnosis

The diagnosis of primary atypical pneumonia rests largely on negative evidence The onset is usually less abrupt than that of pneumococcal pneumonia, the temperature is rarely high and bradycardia is common No leucocytosis is found in the early stages Bacteriological studies are not helpful and there is no response to chemotherapy unless aureomycin, chloromycetin or terramycin are used Radiology shows greater involvement of lung tissue than physical examination would suggest Cold agglutinins develop in about 50 per cent of cases although in some outbreaks either all the patients or none may exhibit them The chief drawback to the diagnostic use of cold agglutinins is that they do not appear until the end of the second week

Serological tests for influenza the psittacosis group and Q fever are negative

Cold Agglutinins

These occur in just over half of all cases In some outbreaks they have been demonstrable in over 90 per cent of cases,

and in others in none They may appear in certain other pyrexial conditions but rarely to a titre of 1 in 32, and a titre of 1 in 64 or over may be taken as evidence of atypical pneumonia The demonstration of a rising titre during the course of the illness is of greater significance than the titre of a single specimen

The nature of the agglutinins is unknown They are apparently associated with the globulin fraction, and will agglutinate human group O erythrocytes at around 0°C They disappear when the serum is warmed to 37°C and reappear if it is once again chilled Blood collected for the cold agglutinin test should not be refrigerated before being sent to the laboratory, as this results in adsorption of the agglutinins by the red cells and a consequent drop in titre

Cold agglutinins are at their peak during the third week and, when they are present, are related to the severity of the infection the more severe the attack the higher is their titre Due to their late appearance they are of little use for immediate diagnosis and their main value is retrospective From the epidemiological standpoint they outrule other virus infections such as influenza and psittacosis, in which they are not found

Streptococcus MG Agglutinins

Some observers have been impressed by the relationship of an 'indifferent" or non specific streptococcus to the ætiology of atypical pneumonia This organism, related to *Str salivarius* has been designated Streptococcus MG It is not now believed to be causative, but agglutinins to it have been demonstrated in significantly higher proportions of those suffering from atypical pneumonia than of the general population

The streptococcus itself can be isolated from the throats of some 12 per cent of normal people, and in the American Commission s experiments it was found in one third of the volunteers before inoculation and in one half afterwards There was however no significant difference between incidence in those who developed pneumonia and in those who did not

It is difficult, therefore, to assess what role this streptococcus may play—as a cause, alone or in conjunction with a virus, or as a secondary invader It does not seem to be causal, for example it is penicillin sensitive whereas virus pneumonias, with the exception of those caused by the *psittacosis* group, do not respond to penicillin If it acts in conjunction with a virus as a primary cause it is an uncommon combination in the pathogenesis of human disease Possibly it merely shares an antigen in common with the infecting virus No experiments on its transmission have yet been undertaken and it is difficult to reach any decision without some such experimental evidence

Opinions vary as to the frequency with which streptococcal and cold agglutinins may be found in the same patient Many hold that almost every patient who shows one agglutinin will also present with the other There is, however, no doubt that the two agglutinins are different—either of them can be removed by absorption without affecting the titre of the other

Epidemiology

As has been seen there is some evidence that any one of several agents may be involved in the production of atypical pneumonia Indeed, the marked difference in laboratory findings between the various surveys leaves little doubt of this, an example is the varied findings in relation to cold agglutinins So far, with the exception of Eaton's virus we have only the scantiest information as to what these agents may be but their existence is unquestionable Our ignorance of their relationship to the common acute respiratory illnesses, which are so frequently and wrongly labelled "influenza," and from which no viruses have as yet been recovered, is one of the great gaps in our knowledge

The incidence of atypical pneumonia amongst the general population is unknown It usually occurs as an endemic condition, small outbreaks being occasionally reported It is considerably more common in the winter months

Infectivity seems to be low, but the disease can be transmitted by infected droplets via the respiratory tract. Contact between cases is often difficult to trace, which is to be expected in any infection with a high proportion of ambulant cases. The incubation period is long and the period of infectivity quite unknown. Second attacks, some time after the first, are known to occur so that no long-lived immunity is produced. These subsequent attacks may, of course, be due to infection with a different virus of the group.

Q FEVER

Q fever, caused by *R. burneti*, was first described in Queensland in 1937. The condition is widespread in many parts of Europe, particularly the Balkans and in the Mediterranean area, in Australia and in the United States. It has recently been described in England. Close contact with cattle as in abattoirs, meat packing plants and dairy farms has been a feature of most outbreaks. Serological evidence of infection was found by the Virus Reference Laboratory, London, in 5 per cent of 800 cases of "virus pneumonia."

Clinical Findings

After an incubation period of about nineteen days the onset is sudden in most cases with severe headache and muscle pains. An occasional case presents merely as "pyrexia of unknown origin" throughout. In the average case the chart shows a high swinging temperature ranging up to 104°F. which lasts for six to ten days and may be accompanied by

consisting merely of a few
for more than a few
changes are found.

atypical pneumonia and shows patchy areas of consolidation at the base One lobe only is usually involved These radiological signs persist until well into convalescence

Gastro intestinal symptoms, apart from those related to the pyrexia rarely occur There is no rash

By the end of a week to ten days the temperature falls by lysis but it may persist for weeks in a few cases Complications are very rare and the mortality rate is low—about 0 2 per cent Aureomycin and chloromycetin appear to be of definite value

Leucocyte counts and other routine laboratory procedures give no assistance in diagnosis The sedimentation rate is raised, as one might expect, in the febrile stage

Pathology

Owing to the relatively low mortality little information is available In the few cases reported there was œdema and congestion of the lungs and hepatization which was confined to one lobe The spleen is enlarged In one fatal case the *rickettsiæ were found widely distributed throughout the* tissues They could be demonstrated in the lungs, spleen and brain

Diagnosis

This is established by the isolation of *R burneti* or by *serological tests* None of the other findings are diagnostic The rickettsiæ may be most easily recovered from the blood during the febrile phase After a few passages they grow readily in mice, guinea pigs or chick embryos The condition is, however extremely infectious in the laboratory, and isolation should not be attempted unless the strictest precautions against infection can be enforced In one laboratory outbreak fifteen of forty nine people working there were attacked as well as one visitor who had merely watched the harvesting of a single batch of infected eggs [f]

Two serological tests are available—agglutination and complement fixation Agglutinins appear first about the tenth

day, and practically all cases will show them at the end of four weeks The test is relatively little used nowadays Complement fixing antibodies are present by the end of the first week and rise steadily until the peak is reached in the third week In either test the demonstration of a rising titre is obviously more conclusive than the result of a single convalescent specimen

Serological tests for influenza and the psittacosis group are negative, nor do cold agglutinins appear

Epidemiology

The most common method of infection is probably the inhalation of dust containing *R burneti*

On the basis of earlier Australian and American work it was thought that ticks played an essential part in the spread of infection In Australia the disease is a natural one in bandicoots amongst which it is spread by ticks These ticks are capable of infecting cattle, and it was thought that cattle ticks feeding on infected beasts became themselves infected and by excreting rickettsiæ in their fæces, contaminated the dust of abattoirs and farms Certainly in both Australia and the United States the disease is largely an occupational one amongst those in contact with cattle

In California where the condition is endemic, an important method of transmission seems to be the drinking of raw milk infected with *R burneti*, and this method of spread has also been reported in England

Recent work has shown a possible link between these two types of spread by the demonstration of the rickettsiæ in the placentas of cows and sheep and also in the naso pharynx and fæces of the latter These could obviously afford a very potent means of contaminating dust, and *R burneti* have been recovered from air samples taken on farms where human cases had occurred

Transmission from man to man is uncommon although it has been reported in a hospital ward In the first English cases infection was contracted at the autopsy on the source

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case The rickettsiæ can, of course be isolated from sputum and urine and these should be carefully disinfected

Antibodies can be elicited by vaccines and have given good results in laboratories working on Q fever but no large scale trials have yet been carried out If satisfactory vaccines would be a valuable protective measure for those at occupational risk

PSITTACOSIS—PNEUMONITIS GROUP

The exact proportion of cases of virus pneumonia caused by members of this group is unknown but it is higher than seems to be generally realized In the United States some 20 per cent, and in England 10 per cent of all cases have been stated to be caused by them Recent work suggests that the latter figure is too high

In dealing with this group we are faced with the same difficulty as with the atypical pneumonia viruses—namely the activation of latent viruses in laboratory stock If anything, the problem is even greater as these viruses are the classicial examples of agents which can remain latent in their hosts for long periods after infection

By far the most important viruses of the group affecting the human are *psittacosis* and *ornithosis* which are closely related to each other Both of them are widespread in nature and infection is usually transmitted to man from different species of birds

Other viruses of this group have also been described as infecting humans They do not appear to be of great significance and little work has been done with them but a few of them may be briefly noted

Meningopneumonitis virus was first isolated in ferrets inoculated with throat washings from human subjects The clinical signs in these cases were suggestive of influenza rather than of atypical pneumonia

Pneumonia Virus of Mice (PVM) was isolated from healthy mice but it has also been incriminated in human cases

Judging by virus neutralization tests infection with it is widespread

San Francisco (SF), *Louisiana* and *Illinois* viruses were isolated from outbreaks in the areas indicated by their names

PSITTACOSIS AND ORNITHOSIS

In the severe outbreak of psittacosis in 1929–30 the disease appeared in twelve different countries and affected 700 to 800 people It resulted in very rigid precautions being taken by public health authorities in many countries to restrict the importation of birds such as parrots and budgerigars At that time the disease was traced to South American parrots, but later work showed that Australian parrots were also infected It is well known that a high proportion of clinically healthy birds are carriers of the virus It may remain latent in them for long periods, only becoming active and being

by the end of which time any infected birds will usually show obvious signs of infection Although irksome, quarantine has proved its value on many occasions Of one batch imported into the United States 97 per cent of the birds had died of psittacosis at the end of the six months quarantine period

This precaution has almost stamped out psittacosis as a public health problem, a fact for which the laboratory worker may be thankful, as it is an extremely dangerous virus to handle and one of the commonest of laboratory infections

Unfortunately psittacosis is itself only part of the problem Recent surveys have shown that ornithosis, closely related and giving the same clinical and pathological picture, is widespread amongst domestic birds in the United States, Canada, Holland, England Ireland and several other countries Pigeons doves, chickens, ducks, gulls, and petrels have all been found infected

To the human patient it is a matter of indifference whether he is suffering from psittacosis or ornithosis, and it is ironical that in spite of the rigid precautions that are taken to prevent psittacosis from entering a country, ornithosis, equally dangerous, should be widespread amongst its indigenous birds The risk to the general population, however, is not great, being confined largely to those whose occupation or hobbies bring them into close contact with infected birds Numerous cases have been reported amongst such persons

Ornithosis was first brought into prominence following an outbreak of a psittacosis like infection in the Faroe Islands where it was traced to fulmar petrels The mortality in the outbreak was 20 per cent, and cases were much commoner amongst those women who were preparing the birds for eating

Clinical Features

After an incubation period of about ten days the onset is sudden with pyrexia, rigors, malaise, sore throat and head ache At first the picture is very similar to an early lobar pneumonia, but cough may be completely absent or at most mild, dry and non productive Sputum is always scanty Physical examination at this stage reveals little, apart from crepitations and an area of dullness on percussion at the base of each lung The temperature, starting at 100°F to 101°F, *gradually rises and remains high* It finally falls by lysis Respirations are normal in rate and the slow pulse is characteristic, rapid pulse or respirations are bad prognostic signs Cyanosis is common and the blood pressure is low Albu*minuria is common at this stage* Insomnia, depression and apathy occur in all except the very mild cases Secondary bacterial infection is uncommon

On X ray extensive patchy consolidation is seen, which *often persists even after clinical improvement* has set in about the third week The leucocyte count is normal or subnormal, but a frank leucocytosis occurs late or during convalescence

Thrombophlebitis may occur as a complication and pulmonary embolism is a common cause of death Relapses have been reported

The mortality rate is high—about 20 per cent—and the prognosis is definitely worse in those over forty years of age

Aureomycin chloromycetin and terramycin are of value in treatment Experimental work with penicillin showed that in many cases its action was merely to mask the infection, and that the virus could be isolated from cases which were clinically recovered Whether this happens with the newer antibiotics is not yet known

Diagnosis

A history of contact with birds is valuable but, of course, inconclusive, as even bird fanciers may develop influenza or atypical pneumonia

The most satisfactory method of diagnosis is by isolation of the virus This is present in the blood for two weeks after onset, and has been found in throat washings, sputum and even vomitus well into the third week In fatal cases the virus should be sought in the lungs and spleen

Material for the isolation of virus may be inoculated intraperitoneally into mice or into the yolk sac of the developing chick embryo

Complement fixation tests are widely used, and can provide a rapid method of diagnosing infection with one of the members of the group, even though not differentiating with certainty between them Complement fixing antibodies begin to appear at the end of the first week and if a rise in titre can be demonstrated in specimens taken within the next few days a presumptive diagnosis can be given A titre of 1 20 in patients clinically suggesting psittacosis may be taken as positive

Cold agglutinins have been found but rarely and serological tests for influenza and Q Fever are negative

Pathology

The areas most affected are the lungs, bronchi, and trachea On examination with the naked eye the lungs appear to be uniformly consolidated but microscopical examination shows that, in fact, consolidation is lobular in type and not evenly distributed Alveoli containing air can still be found in the midst of consolidated areas, a fact which tends to mask the clinical signs of consolidation A variety of cells are seen in *the alveoli—red blood cells, lymphocytes, macrophages, and* a small proportion of polymorphs In suitably stained preparations clumps of elementary bodies may be found in the cells of the alveolar exudate There is also a marked desquamation of the bronchial epithelium

In other organs fatty degeneration is frequently found, particularly in the liver where there may also be a focal necrosis in the centre of the lobule

Epidemiology

Infection with viruses of the psittacosis group is widespread *in nature Their attribute of persisting for long periods* without any obvious clinical, or even serological, signs of infection on the part of the host, renders it likely that many species are infected

Amongst birds, infection is caused by direct contact, droplets or infected droppings Young birds are infected in the nest or, if they escape there, immediately afterwards by mixing with other birds A variable proportion of these die on their first exposure to the virus The survivors gradually cease to shed virus and by the end of some three months are clinically well, but if sacrificed then they will be found to have an enlarged spleen from which the virus can be isolated Breeding females frequently become active distributors of virus thereby passing on the infection from generation to generation

Infection may spread from birds to man Relatively close contact is necessary, and the vast majority of cases have

occurred in those whose occupation involves the handling of birds They are infected through their association with infected birds, or by contact with the feathers or excreta The occupational risks do not seem to be sufficiently realized, although there are many instances of such spread The same arguments, of course, apply to those keeping psittacine birds as pets

Man to man transfer is alarmingly easy, and the risk to those who have to nurse psittacosis patients is very real as hospital outbreaks show In one instance twenty six cases occurred amongst the hospital staff with thirteen deaths, and in another nineteen cases with eight deaths Ornithosis is less infectious but even more lethal

Rigorous isolation of all cases for a considerable period is necessary as the virus may be excreted by them for at least four weeks All discharges from the patient must be ade quately sterilized Enquiries as to the source of the infection should be immediately set on foot In these islands the quarantine measures in force have stood the test of time, but there is always the danger that thoughtless smuggling of birds may introduce infection, apart from the risk from native species In California control broke down completely due to widespread smuggling

Persons fully recovered from an attack are generally thought to be immune but this may be due to persistence of the virus as happens in birds A case has recently been discovered actively shedding virus in the sputum eight years after a severe attack, suggesting not only that the virus may persist, but also that it may not remain completely latent

Tests of vaccines, phenolized or with dilutions of active virus, have shown that antibodies are produced by them Their significance or value in combating infection is unknown

Chapter Seven

LYMPHOGRANULOMA INGUINALE

(Lymphogranuloma venereum, *Lymphopathia venerea*)

Lymphogranuloma inguinale is a virus disease of lymphoid tissue Its transmission is usually venereal, and the condition occurs throughout the world

CLINICAL FEATURES

The incubation period varies from three or four days to three or four weeks It is difficult to estimate in many cases as the primary lesion may be so mild as to pass unnoticed *This most frequently presents in males as a small vesicle on* the glans penis In women it may be found on the labia, but often occurs on the vaginal wall or even on the cervix Alternatively, the lesion may be intra urethral and a non specific urethritis results The vesicle gives way to a shallow ulcer which heals within a few days

The second stage follows within one or two weeks when the *regional lymph glands become enlarged and painful* At first they are discrete, but later they become matted together due to the extensive suppuration and sinuses are *formed which* drain for weeks or even months A small proportion of cases heal spontaneously and do not go on to pus formation

Later the picture is dominated by a very extensive fibrosis around the affected lymph glands with induration and dis coloration a condition known as "esthiomène" There is often some degree of elephantiasis involving the external *genitalia and proctitis and rectal stricture gradually develop (genito anorectal syndrome)*

The clinical picture varies considerably *according to the* sex of the patient In the male the inguinal glands are

involved with the formation of buboes and sinuses as described above In the female, however, the lymphatic drainage is to the pelvic glands, and the pathological process of suppuration and fibrosis occurs within the pelvis Should the primary lesion in the male be intra urethral the pelvic glands, and not the inguinal, are affected

Occasionally a generalized rash is seen, and rarely a meningo encephalitis has been reported

The serum proteins are raised due to an increase in the globulin fraction Otherwise there is little characteristic in the clinical laboratory findings With the onset of suppuration a polymorphonuclear leucocytosis is found

TREATMENT

Good clinical results have been obtained with sulphadiazine in the primary and secondary stages, but it is more than doubtful if a complete cure is possible with the sulphonamides *Penicillin seems to be of little value* *Aureomycin* in a dosage of 25 mgm /kilo orally, or 20 mgm /kilo intramuscularly has given promising results Chloromycetin is effective in experimental infections, but no reports of its use in human cases are yet available Elementary bodies can, however, still be demonstrated in the tissues of animals after clinical cure

It is difficult to assess the value of chemotherapy or of the antibiotics in these cases There are two major factors involved and we know little of either The first is the method whereby the drugs act on the virus, and the second is the criterion of cure

Even *in vitro* the sulphonamides are virucidal only in very high concentrations In experiments with mice clinical signs e g meningitis in mice inoculated intracerebrally, may be suppressed but the virus none the less persists in a high proportion of cases Whether or not the newer antibiotics such as chloromycetin or aureomycin are virucidal or merely suppressive still remains to be seen but from what has

already been said of chloromycetin it would seem quite possible that their action is one of suppression rather than of cure

PATHOLOGY

The primary lesion shows the characteristic histological features of ulceration

In the secondary stage, foci of reticulo endothelial cells are at first prominent in the affected lymph glands These give way to small abscesses which gradually enlarge and destroy the whole structure of the glands, until they finally *become a matted, necrotic mass Simultaneously extensive* fibrous tissue is laid down, which, when it contracts, produces the lesions of the tertiary stage

Inclusion bodies of two kinds are found in infected cells Large basophilic bodies, first described by Gamna and Favre and named after them, may be seen in the cytoplasm of histiocytes and mononuclears These vary in size from 1 to 4 μ, *and are nuclear in nature They are not virus particles,* but they have a diagnostic significance

The second type, Miyagawa's "granulo corpuscles," are small basophilic bodies of about 0 3 μ in diameter and are virus elementary bodies They stain readily with Giemsa, and are found most easily in the cells surrounding an abscess

LABORATORY DIAGNOSIS

The virus is abundantly present in all the lesions and infection can be demonstrated not only by isolation or sero logical procedures, but also occasionally by *microscopy* It is present in the cerebrospinal fluid in cases of meningo encephalitis, and in the fæces in cases with proctitis

Isolation of the Virus

For isolation of the virus, pus from the buboes is inoculated into experimental animals or into the yolk sac of the develop ing chick embryo *The most susceptible animals* are monkeys

and mice After intracerebral injection they both develop a meningitis within seven to fourteen days and elementary bodies can be demonstrated within infected cells Guinea pigs may also be used the injection being made into the groin but they cannot be infected so readily as the other two species

Several passages may be required before the virus becomes established in chick embryos and animal inoculation gives more rapid diagnosis

Serological Tests

Frei Test This skin test becomes positive between two and six weeks after infection The original material used by Frei consisted of heated pus from a bubo injected intra dermally but infected yolk sac material from chick embryos is now generally used The test must be carefully controlled and the size of the papule formed is of importance That resulting from the test material should be not less than 7 mm in diameter while that from the control of normal yolk sac should have a diameter of not more than 5 mm The result is read after forty eight hours

With the methods of treatment available until the advent of aureomycin and chloromycetin the test remained positive for life What effect they may have on it is not yet known

Complement Fixation Test This is more sensitive than the Frei test and may become positive earlier It must be remembered that a positive result merely indicates infection with a member of the psittacosis--lymphogranuloma group of viruses, and that it is not specific On the other hand provided its limitations are understood it is an extremely useful test in the diagnosis of infection with the members of the group

The antigen most widely used is prepared from infected yolk sacs and using it a titre of 1 16 may be regarded as suggestive of infection A rising titre is of course more significant than the titre however high of a single specimen Non specific positive results have been reported in syphilitic patients but it has also been suggested that such cases are

in fact, early cases of lymphogranuloma Be that as it ma positive results to low titres in such patients should be inte preted with caution unless the test remains positive afte adsorption of the serum with Kahn antigen

The complement fixation test remains positive for as lon as the virus is active in the tissues

The Wassermann and Kahn reactions should also be carrie out on all patients, as double infections, with syphilis an lymphogranuloma are relatively frequent

EPIDEMIOLOGY

Lymphogranuloma inguinale has a world wide distributio and cases have been reported from every quarter of the globe It seems unquestionable that many cases are missed, especially in temperate zones because the practitioner does not con sider the condition in his differential diagnosis

Sexual intercourse is the most common method of spread but occasional non venereal infections are met with in doctors and laboratory workers through contact with infected pus

The virus has, on occasion, been isolated from lymph glands several months after apparently adequate chemo therapy

PROPERTIES OF THE LYMPHOGRANULOMA—PSITTACOSIS—PNEUMONITIS GROUP

The discussion of the clinical features and the epidemiology of psittacosis has been included in Chapter Six It is prefer able however, to describe the characteristics of the group in one section because of the close relationship and many similarities between its members

Infection with members of the group—either clinical or latent—is widespread in nature, man, animals and birds being attacked The more important members of it may

be enumerated as follows—lymphogranuloma inguinale psittacosis, ornithosis meningo pneumonitis human pneumonitis e g SF, Illinois and Louisiana viruses, feline pneumonitis (Baker) and pneumonia virus of mice In addition the viruses of trachoma and inclusion conjunctivitis bear a very close relationship to the group

The members of the group are usually accepted as occupying an intermediate position between the viruses and the rickettsiæ and it is doubtful if they can be considered to be true viruses They are filtrable and unable to grow except in the presence of living cells but may be easily stained by simple stains such as Giemsa or Castaneda and examined under the ordinary microscope Their morphology is so similar that they cannot be differentiated microscopically Some of them are susceptible to sulphonamides and antibiotics A few of them produce an endotoxin

Inclusion bodies which are aggregates of virus particles or elementary bodies are found in infected cells These are basophilic when stained by either of the methods just mentioned and the term basophilic viruses is often applied to the group The elementary bodies are reported as varying in size from 250 mμ to 450 mμ depending on the particular virus Some of the variations may arise from the methods used to estimate the size rather than from differences amongst the viruses

A life cycle all of which takes place inside infected cells has been proved or postulated for the members of the group It may be briefly described as follows After entering the cytoplasm of the cell the elementary body enlarges to form an initial body which is about 1 μ in size These initial bodies divide and occupying more and more of the cell form what is termed a plaque or morula several microns in size This plaque is accepted to be a colony of virus particles On ordinary microscopical examination it may appear structureless but by special methods it can be shown to be composed of small particles embedded in a matrix which is probably cellular in origin Ultimately the cell ruptures and releases

numerous elementary bodies, which in their turn infect other cells and repeat the cycle

Not all the viruses of the group are as well characterized as are psittacosis and lymphogranuloma Trachoma and inclusion conjunctivitis are very similar microscopically, they show some sensitivity to the sulphonamides and con

mation is available about them

These basophilic viruses are very closely related antigenically The serological diagnosis of infection with some member of the group can be readily made, but the demonstration of which one is actually involved is still a matter for the research laboratory Even when one of them has actually been isolated elaborate investigations to identify it may still be required

The most valuable single test for identification is the virus neutralization test, which demonstrates sharp differences amongst these viruses The data gained from this test, which is still an experimental procedure, fit in well with the other tests which have been used These include sulphonamide sensitiv

tests

under i

definite conclusions can be drawn from the results obtained The criteria are the degree of success in infecting an animal by any given route, and whether or not the animal survives the infection Should it survive, the establishment of a carrier state is another point of differentiation For instance, lymphogranuloma virus will infect mice if inoculated intracerebrally but not intraperitoneally, while psittacosis will infect by the latter route

In view of the marked tendency of these viruses to persist as latent infections even after apparent cure, the identification of the specific infection may be of great importance, clinically and epidemiologically

CHAPTER EIGHT

MEASLES
(Morbilli)

MEASLES is the most prevalent and probably the most infectious of the exanthemata It has always carried a high mortality from respiratory complications in childhood. Recent advances in prophylaxis and treatment have materially reduced its dangers, but it still remains a serious condition in children—more particularly in those under three years of age

CLINICAL FEATURES

If it be calculated from the date of exposure to the appearance of the rash, the incubation period is twelve to fifteen days Occasionally, longer periods—up to twenty one days —have been reported It may be lengthened in those who have received serum or gamma globulin for the attenuation of an attack An "illness of infection" occurs in a number of children a few hours after exposure to infection, catarrh, respiratory symptoms, fever and even a morbilliform rash last for some twenty four hours

The illness itself begins with a well defined prodromal stage which lasts three to four days before the appearance of the rash It is characterized by pyrexia, catarrh involving the whole respiratory tract and injected conjunctivæ Koplik's spots, which are found in practically every case of measles and are pathognomonic, first appear two or three days before the rash as small pin point specks with a surrounding red areola, on the buccal mucous membrane They are found initially opposite the lower molars, and at about the time the rash appears they spread over the mucosa giving it a fine

granular appearance Prodromal rashes of an erythematous or scarlatiniform nature are common, but last only a very short time Virus is present in the blood and the naso pharyngeal and buccal secretions during this stage, and infectivity is now at its height

The patient becomes gradually more ill until the true rash appears It is first seen behind the ears and on the forehead about the fourth day, and thence spreads rapidly over the trunk and limbs Originally macular, and then papular in type, it becomes dusky within twenty four hours—occasionally so deep in colour as to seem hæmorrhagic It tends to become confluent in some areas, giving the skin a blotchy appearance At this time the patient is very ill with a high temperature, ranging up to 103°F, rapid respirations and pronounced catarrhal symptoms Bronchitis is almost invariably present all through the prodromal and eruptive phases

Once the rash is fully developed—a process which occupies about forty eight hours—the temperature falls quickly and the patient s general condition rapidly improves The rash then fades in the order of its appearance and leaves behind a characteristic brown discolouration of the skin This is frequently followed by desquamation of the affected areas

Complications

Measles, by itself is not often a dangerous disease but the complications which may arise are so numerous and so frequent as to make it a serious infection in childhood Until quite recently the mortality was high principally because of secondary respiratory infections In hospitals cross *infection leading* to complications is particularly frequent Oiling of bedclothes and floors may be of great value in diminishing its incidence, and in making it possible for measles patients to be nursed in hospitals without serious *sequelæ*

The most important single complication, and the cause of most deaths, is broncho pneumonia which may be present

as early as the stage of eruption Bronchiectasis is a common end result in those cases which recover from it Occasionally a true virus pneumonia occurs but the commonest organism involved is the streptococcus, staphylococci, pneumococci and *H influenzæ* are also frequently isolated from these cases Otitis, laryngitis and conjunctivitis may also follow an attack of measles, and the first of them is still common Even though chemotherapy and antibiotics have materially reduced the danger of these complications, the disease remains serious in under nourished or debilitated children If any of these are at risk there is no excuse for not protecting them by passive immunization If such children were adequately protected the mortality in measles would be negligible

Encephalomyelitis which closely resembles, clinically and pathologically that following vaccination and the other acute infectious fevers, is a rare occurrence It usually appears as the rash is beginning to fade The sequelæ seem rather more severe following measles The mortality rate varies between 10 and 30 per cent and the prognosis for the survivors is not good as permanent mental or physical disability follows in some 50 per cent of the survivors The condition is possibly due to the measles virus itself which has been isolated from the brain of one fatal case

EPIDEMIOLOGY

Except in isolated communities the highest incidence of measles is in the under five age group Practically every child is born with a passive immunity derived from a mother who has had the disease herself in childhood This wanes at about the end of six months Measles is rare, therefore, before some nine months of age but from this until three years the incidence rises steeply It then falls away but practically every child has been attacked by the age of five Should measles occur in infancy the baby is frequently infected by the mother who has never previously suffered an attack herself

Such is the picture of measles in cities in populous countries, but it is very different in many rural areas, and indeed in isolated communities the epidemiology bears no resemblance at all to it The age distribution depends almost entirely on the time between epidemics, and the picture given is based on the behaviour of measles in the large cities of Great Britain In them an outbreak may be expected every two years The virus itself is always present in the community but the soil for an outburst is not always there, and before an epidemic can take place there must also be a pool of susceptible children These are principally the children who have lost their inherited passive immunity since the last epidemic Some children may also have acquired a slight degree of immunity by contact with cases which, however, is not sufficient to protect them against a clinical attack of measles a year or two later

In rural areas the picture is modified by several factors In the first place there are not so many children to form the pool of susceptibles Secondly, the opportunities for the transmission of the virus are not so good, and thirdly, there is less virus present in the community The time between epidemics is therefore longer, and children may not contract the disease until a later age than those in the cities and large towns In the Wensleydale villages, for instance, nine years elapses between epidemics

When we come to examine isolated communities such as those living on remote islands the picture is different again, although improved methods of communication are nowadays modifying it The classical examples were outbreaks in the Faroe and the Fiji Islands where the disease had not appeared for many years and adults were therefore as susceptible as the children When measles was introduced both were attacked with a high mortality at all ages This is the typical pattern of a new disease striking any community

Measles therefore can occur at all ages and in all climates The typical picture in crowded communities is due to a widespread incidence of the virus, which has ample

opportunities for transmission and emerges in epidemic guise when enough susceptibles have accumulated, a process which takes about two years In more sparsely populated areas it takes longer, susceptibles are not so numerous and the facilities for spread are not so good When we come to the really isolated islands the virus, on its rare visits, either kills or immunizes all the susceptibles In doing so it leaves itself no reservoir from which to initiate another outbreak, and it therefore dies out The population will then be free of the disease until a fresh introduction of the virus When that occurs, maybe years afterwards everyone will be susceptible except those still living who survived the previous outbreak

This lifelong immunity after an attack is a dominant factor in the epidemiology of measles In densely populated countries almost every adult is immune due either to a frank clinical infection or to sub clinical attack These must be relatively frequent probably, indeed, more frequent than the former This immunity is solid and on occasion has been demonstrated in spectacular fashion For instance, in the Faroes outbreak of 1846 it was shown to have lasted sixty five years in the survivors of the previous epidemic Second attacks are very rare but a few have been reported

Most cases in temperate climates occur during the winter and early spring

The virus is abundantly present in the oral and naso pharyngeal secretions during the prodromal period, and also for some forty eight hours after the appearance of the rash At this time infectivity is at a peak and it is almost impossible to prevent the disease spreading to susceptible contacts of the sick child If the indications point to it they can of course be protected by the prompt use of one of the methods of sero attenuation which are described below

Infection is disseminated by droplets which may travel a considerable distance inside buildings as for example in class rooms These are the most important source of infection Fomites may play a part but they are not of great significance as the virus does not survive outside the body for any length

of time Nevertheless, articles of clothing or toys which have been recently contaminated by infectious secretions may be dangerous for a short time The virus is also present in the rash but this is of little importance in the spread of infection

A good instance of the extreme infectivity of the disease is given by Pickles, when he describes the case of the 'long window " A boy, ill in bed, infected his aunt who had never seen him during her stay in the house, and had in fact left the house on the morning after he had been sent to bed On investigation it was found that one long window served two rooms on different floors, in such a way that one room had a direct connection with the other, because, at the window, there was a gap in the flooring The patient was in the upstairs room and his aunt sat at meals directly below the gap Twelve days later she developed measles

CONTROL

The general rule is to isolate all cases for two weeks from the appearance of the rash, but it would seem that this time is longer than necessary Ten days or even a week are probably ample

Contacts are sent into quarantine seven days after exposure and are kept there for a further ten days to a fortnight These rules are now subject to revision in the light of experience with serum and serum products—depending on whether they are used for the prevention or the attenuation of an attack

Special care must be taken to protect children under three and those whose general health is not good In them the risk of complications is appreciably greater than in the average child

The closing of schools is of little value in aborting epidemics

Passive Immunization

There is nowadays no excuse for not combining immunization with isolation and quarantine—indeed in many cases

it will replace the latter On epidemiological grounds immunization is of far greater importance than the older procedures which are difficult to enforce and are of doubtful value due to the infectivity of the disease Also it can be easily and rapidly carried out and for both the practitioner and the patient it robs measles of much of its former dangers

It has been known for many years that the serum of those who are convalescent from or immune to measles will have a modifying effect on the course of the disease As the morbidity in childhood is so high this means in effect that the serum of almost every adult in any large centre of population can be used for this purpose The advances in recent years have been directed mainly towards finding improved and more efficient materials for injection The basic principles of this form of protection remain the same

Once it has been decided to use serum or any of its products in dealing with contacts either of two objectives may be attained It is possible to prevent the attack completely or to attenuate its severity In general it is far better to aim at attenuation This gives rise to a mild attack of measles lasting only some twenty four hours and the patient has a subsequent permanent immunity just as after the classical disease

In many cases it is desirable to prevent an attack alto

older or is better able to withstand its effects In the conditions of the present day it may be taken that unless it lives a completely isolated existence every child will contract measles at some stage Attenuation could then be produced after a subsequent exposure

When an attack is suppressed by passive immunization it must be remembered that as in all examples of its use this type of immunity wanes quickly and the child is again fully susceptible to infection a few weeks later

The substances which may be used are outlined below and certain general principles apply to all of them The timing of

the prophylactic dose is of great importance If prevention is desired it must be given on or before the fifth day after exposure to infection To attenuate an attack either of two courses may be followed *The first is to give the injection after the fifth but not later than the ninth day*, after the ninth day no effect can be expected The alternative is to give on or before the fifth day half the dosage which is recommended for prevention

It is important that these time limits should be adhered to Also, the rationale of these methods should be understood, even though serum was used empirically with good results for many years before any comprehensive theory of the pathogenesis of the incubation period was put forward

Pathogenesis of Measles *It appears very probable that* after its implantation in the body the virus of measles multiplies first in some internal site possibly, according to Fenner, *in the spleen Thence, during the incubation period the virus* is disseminated through the blood stream before it localizes in susceptible cells in the mouth, nasopharynx and skin If such a stage of viræmia does occur, it is obvious that no injection of serum will abort the clinical disease *unless it is* given soon after exposure The virus has lodged in the cells of the skin, for instance, several days before the onset of the rash, and no means of prevention known to us can be of value once it has got into them The rash itself does not appear until the amount of virus in the skin reaches its maximum concentration, and it represents a late stage of infection—not an early one Similarly it is not correct to speak *of the* prodromal stage as a stage of invasion " Invasion has taken place several days earlier and before any symptoms arise

If the injection of serum is timed to produce a high concentration of antibodies in the blood stream before the stage of viræmia occurs, then the virus is destroyed or neutralized by the antibody and no invasion of the skin or other sites will take place Hence no clinical attack of measles *will occur* Should they be present in smaller amounts due either to smaller dosage or later inoculation, then the virus present

will not be completely neutralized and a mild attack will develop

This concept of the pathogenesis of the exanthemata has not been proved beyond all doubt but there is a considerable amount of experimental evidence in its favour In the case of measles it certainly provides a reasonable explanation both of the stages of infection and the mode of action of sero prophylactic preparations Also it demonstrates clearly why even large amounts of convalescent or other immune human serum would be quite useless in the treatment of a case

Serum Prophylaxis

The following agents are available for the prevention or attenuation of measles —

CITRATED WHOLE BLOOD

This is usually obtained from the relatives of the child and is injected intramuscularly As might be expected its anti body content is low and its value s very questionable A further disadvantage is that tests for the ABO and Rh blood groups must always be carried out

ADULT SERUM

Blood is collected from several donors but pools should be kept small to minimize the risk of serum hepatitis After Wasserman tests have been carried out the serum is sterilized by filtration and stored in ampoules for use It may also be dried and issued in ampoules in that form being reconstituted just before use

No properly controlled data as to the optimum dosage are available and the amounts given are quite empirical The dosage recommended is 10 ml for children under three years of age and 20 ml for older children The injection is given intramuscularly

The value of normal adult serum in giving complete pro tection is doubtful but some degree of attenuation is usually

obtained if it is used within the first five days from exposure to infection

CONVALESCENT SERUM

Blood is collected in the same manner as for adult serum but, as the name implies, it is obtained from those who are in the early stages of convalescence In practice it is collected during the second week, after the temperature has subsided, and when antibodies to measles are present in high concentration It is difficult to obtain because, as a rule, only adult convalescent patients are suitable donors, and the number of these is naturally very small

No information is available as to the optimum dosage 5 ml is usually given by intramuscular injection to the under three years group, and 10 ml to the older children, before the fifth day when prevention is required The same doses given before the ninth day will attenuate an attack, or alternatively, half of the dosage specified may be administered before the fifth day This is preferable, as the supplies of convalescent serum are so limited

The chances of serious sequelæ such as serum hepatitis must be negligible Unfortunately, with one batch used in London in 1949 five fatalities were described amongst children who had earlier received prophylactic measles injections While this tragic episode, the only one of its kind reported, should not be permitted to detract from the value of the procedure, it is fortunate that gamma globulin, free from such risks, is now generally available

PLACENTAL EXTRACT ("HUMAN IMMUNE GLOBULIN")

This preparation is not widely used, gamma globulin being preferred It is liable to give rise to reactions, some of which may be severe The amounts given are 2 ml intramuscularly in children up to two years of age, a further 0 25 ml for each year of age is given in older children up to a maximum of 4 ml

GAMMA GLOBULIN

This is the substance of choice and it has given extremely successful results It consists of the gamma portion of human globulin, that is the fraction containing the highest proportion of antibodies In terms of concentration of the antibodies it represents a twenty five fold increase over the original plasma from which it is prepared

The recommended dosage is 0 1 ml per lb of body weight to be given as usual, before the fifth day for prevention, 0 025 ml per lb body weight is used for attenuation A system of dosage based on age has also been recommended It is given by intramuscular injections

No cases of serum hepatitis have been reported following the use of gamma globulin, on the contrary it has been recommended for the prophylaxis of hepatitis, if given early in the incubation period in a manner analogous to its use in measles

Active Immunization

Experiments with vaccines prepared from chick embryo cultures have been carried out with some degree of success It is not yet known how long the immunity which they produce will last, and it is too early to say how valuable this technique may prove to be On general principles of course, active immunization is much preferable to even the most efficient method of passive immunization and now that sound methods of attenuation are available many of the risks which previously attended attempts to produce active immunity may be disregarded

PROPERTIES OF THE VIRUS

Measles virus survives at room temperature for more than twenty four hours and at —72°C for four weeks It is killed in fifteen minutes at 55°C It will resist 50 per cent glycerol for three months, and ether at room temperature for forty minutes

A number of animals are susceptible to the virus mainly monkeys, on which most experiments have been carried out On infection they develop pyrexia, Koplik's spots and a rash Growth occurs in the chick embryo but no characteristic lesions are produced

The size of the virus is unknown, but it passes Berkefeld N and Seitz EK filters

Chapter Nine

RUBELLA

(German Measles)

Rubella has always been regarded as one of the mild infections of childhood, and has been little studied because of the low infectivity and negligible mortality of the condition The illness is mild in both children and adults, and were it not for its effects on the fœtus, when an attack occurs during pregnancy, the virus would be of little significance These sequelæ were first described in 1941 by Gregg, who reported a series of congenital defects in the infants of mothers who had contracted rubella in the early months of pregnancy This finding has been amply confirmed since then and has aroused considerable interest, but in spite of the intensive work to which it gave rise little is yet known of the properties of the virus

CLINICAL FEATURES

The incubation period is usually between fourteen and eighteen days There may be a prodromal stage of malaise in some cases but it seldom lasts for more than a day In the majority the disease begins with the appearance of the rash This is first seen on the face and spreads rapidly, to involve the trunk and limbs within twenty four hours It consists of small discrete macules, pink in colour, which seldom become confluent except in severe cases, and even then only on small areas of the skin It fades from above downwards in the same order as it appeared, and does not last for more than one or two days Occasionally it is even more fleeting, which may make diagnosis difficult but it should be typical on some part of the body when the patient is examined Quite possibly

it will have completely faded from the face, and even from the trunk, at the time of examination

Apart from the rash the most prominent clinical feature is a stiffness of the neck, arising from enlarged and tender lymph glands in the posterior triangle, a sign which may be present as early as the prodromal phase In some cases, particularly those in whom the condition is relatively severe, this forms part of a generalized adenitis in which any or all of the lymph glands may be involved

Constitutional signs are slight The fauces are congested and a mild nasal catarrh is found The temperature may rise to 100°F or 101°F for twenty four to forty eight hours By the end of this time, the rash is fading, the glands have resolved in most cases, and the temperature returns to normal

The diagnosis is readily made during epidemics, and in those cases in which the appearances are typical Occasionally *the distribution of the rash at the time of seeing the patient* may be such as to suggest measles or mild scarlet fever, but the subsequent course of the attack outrules them Serum or drug rashes can be precluded by the history The blood picture and the distribution of the rash, as well as a negative Paul Bunnell test, distinguish rubella from infective mononucleosis

The peripheral blood shows a leucopenia in the early stages which has changed to an absolute lymphocytosis by the end of five days Turck cells are frequently found in stained films

COMPLICATIONS

Encephalomyelitis is a rare complication The mortality is about 25 per cent, but only occasional reports of the path ology are available *Demyelination has not been described*

FŒTAL DEFECTS

Congenital anomalies were first described by Gregg in Australia, and the causal relationship between maternal

rubella during pregnancy and these defects has since been confirmed by other workers Within the past few years the condition does not seem to have been described so frequently This appears to lend weight to the suggestion first put forward to account for the lack of reported cases prior to 1940 that the strains of rubella virus prevalent in that and the immediately following years may have been of a more virulent type than usual No work however has been reported on this particular aspect and in spite of some interesting pointers it still remains conjectural

In the series described by Gregg there were 78 cases of congenital cataract, more than half of which also showed congenital heart defects on examination Upon investigation of these cases it was found that no less than 68 of the mothers of these children had an attack of rubella during the first or second month of the pregnancy—at a time when the disease was epidemic Later work principally in Australia and the United States showed that maternal rubella was most dangerous for the fœtus during the early months of pregnancy In such cases congenital defects could be expected in a high proportion of the infants born subsequently

The most common fœtal defects are congenital heart disease cataract deaf mutism and microcephalus All three embryonal layers are involved and the virus appears to pass the placenta and act directly on the fœtal cells There is a definite correlation between the period of the pregnancy in which rubella occurs and the subsequent anomalies As might be expected the virus acts with greatest violence on those cells which are most actively developing at the time of the attack During the first month ocular defects may be expected Similarly in the second month cardiac defects and in the third deafness could be forecast Stillbirths may also be attributed to the action of rubella on the fœtus

After the third month of pregnancy the incidence of defects falls sharply In general it may be said that the later the attack the less likely it is to affect the child and an attack in the last few months is unlikely to leave any sequelæ

Estimates of the number of children affected vary considerably At one time it was thought that there was a virtual certainty of subsequent defects following rubella in the first two months of pregnancy, and a 50 per cent chance in the third month More recent work puts the incidence at between 20 and 30 per cent in the first three months

It is obvious that all possible steps should be taken to protect pregnant women from rubella, but the means of doing so leave much to be desired A high proportion of them will, of course, have had the disease in childhood, and as one attack confers a lifelong immunity need not be further considered The others should be protected against exposure to a known case as far as possible, as once they have been infected little can be done to abort an attack Convalescent serum may be of value Ordinary gamma globulin, as used for measles prophylaxis, has little effect, unless it is prepared from the plasma of those convalescent from rubella Either of these, and particularly the latter if available, should be injected as soon as possible after exposure

EPIDEMIOLOGY

Rubella occurs in epidemic form usually in the spring and early summer The highest incidence is in childhood but young adults are frequently affected The virus is present in the nasopharynx and the blood during the acute stage, and infection is spread by droplets The patient is infectious for a short time only that is in the prodromal stage and probably for a day or so afterwards

In view of the mildness of the condition no rigid control measures would seem to be indicated for children Indeed, as second attacks are very rare, it would seem preferable that females at least should suffer an attack in childhood

PROPERTIES OF THE VIRUS

Apart from the fact that the infectious agent is filtrable little is known of its properties

The only susceptible experimental animal is the rhesus monkey, which can be infected by the inoculation of throat washings or of blood collected in the acute phase No lesions are produced in the chick embryo by the inoculation of such specimens, but material from inoculated eggs will induce the disease if subsequently injected into monkeys

There is apparently no relationship to the virus of measles, and no cross immunity occurs

Chapter Ten

VARICELLA
(Chicken pox)

As a rule chicken pox is a very mild disease, but by virtue of its extreme infectivity it is one of the most important diseases of childhood It sometimes, and especially in severe cases, resembles smallpox and its accurate differentiation may then be a matter of urgency This is particularly so during small pox epidemics, when varicella is frequently made notifiable so that the diagnosis may be verified as quickly as possible Students, practitioners and public health officers should therefore be conversant, not only with the clinical differences between the two conditions, but also with the diagnostic laboratory facilities available Few practitioners see enough cases of smallpox to be at all confident in their dealings with it, and an atypical case of chicken pox can give rise to considerable anxiety before the correct diagnosis is established

CLINICAL FEATURES

The disease begins between twelve and twenty one days, usually fourteen to sixteen days, after exposure and in the majority of cases the first sign is the appearance of the rash In some there is a prodromal period lasting two to three days with malaise, anorexia and headache These cases frequently show a scarlatiniform prodromal rash, which fades either with the appearance of the pocks or shortly afterwards

Constitutional signs are very slight and many cases show only a negligible rise in temperature—others may show none at all There may be a small spike caused by pustulation if secondary infection is marked If the eruption is very severe,

an occasional occurrence only, there is a definite pyrexia and the patient may be then extremely ill for a time These are the cases in which a diagnosis of smallpox is often made

The irritation caused by the rash may be very marked—it is often the only symptom which requires alleviation Care should be taken to minimize secondary bacterial infection Treatment is therefore symptomatic but there have been recent reports of good results following the administration of aureomycin and chloromycetin

Severe varieties of chicken pox such as *gangrenous bullous* or *hæmorrhagic* have been described but they are very uncommon

Specific Eruption The specific eruption is first seen as a macule and it goes through all the stages to the pustule with great rapidity Unlike smallpox those cases with prodromal symptoms show no clinical improvement when the rash appears It shows first on the trunk and spreads quickly to the face in either of these situations the eruption may be severe Pocks are found on the limbs but in contradistinction to smallpox are most numerous on the upper arm and legs and are sparse on the extremities The centrifugal distribution characteristic of smallpox is not seen Should pocks occur and they occasionally do on the palms of the hands or the soles of the feet they are scanty and superficial and disappear in the course of a day or so There is no tendency for the rash to avoid concavities or the skin flexures It may be visible but only for a short time on the fauces and the buccal mucous membrane Changes in its distribution due to previous irritation or to trauma may be found

As many as five successive crops of lesions may appear during an attack and this combined with the speed of their development from macule to pustule means that in any one area of the skin all stages of the eruption may be seen simultaneously Vesicles succeed the macules within a matter of hours and have themselves become pustules twenty four hours later The latter dry forming a scab which desquamates in the course of a week or ten days

The vesicles of chicken pox are usually so superficial that they seem to be lying on the skin Many of them are oval in shape with their *long axis parallel to the skin folds in their* neighbourhood They are unilocular and collapse completely when pricked Even the slight trauma caused by the pressure of clothing may be enough to rupture them a brown scab then forms over the raw area As they develop their contents gradually become cloudy and then frankly pustular

Elementary bodies are present in the vesicle fluid but they *are few in number and are difficult to stain by methods which* will readily demonstrate variola elementary bodies

Complications rarely occur numbering only about 0 2 per cent of all cases in one large series Minor degrees of pyogenic skin infection are common in spite of all attempts to prevent them

Encephalomyelitis which is similar in type to that *following the other acute infections* has been reported It comes on four to ten days after the onset of the disease with vomiting ataxia nystagmus and tremors The prognosis is good in these cases following chicken pox

LABORATORY DIAGNOSIS

Laboratory diagnosis is seldom required except in severe cases during a smallpox epidemic when it is of great importance With one possible exception there are no positive tests for varicella infection and the diagnosis is based on the clinical findings and the fact that all the tests for variola are negative

In the early stages films may be prepared from vesicle fluid and stained by e g Paschen s or Gutstein s method While in variola the elementary bodies are seen in profusion in varicella they are very scanty and are only stained with great difficulty As a positive diagnostic method for varicella it is of little value

Serological tests using variola or vaccinia antigens are completely negative in the case of chicken pox—unless of

course, the patient has been recently vaccinated Complement fixing antibodies have been demonstrated in cases of varicella using dried crusts as the antigen, but the test cannot be regarded as a routine procedure

Unlike variola or vaccinia, varicella virus has not been isolated in the chick embryo, so that no growth will occur after the inoculation of vesicle fluid on to the chorio allantoic membrane This is another important point in differentiating between chicken pox and smallpox, which grows readily and gives rise to characteristic lesions

EPIDEMIOLOGY

There is little seasonal variation in the incidence of varicella, although it is somewhat more prevalent during the winter months Most cases occur in childhood but young adults may be attacked In these the condition is often severe

Chicken pox is contracted by the inhalation of infected droplets and cases are infectious some twenty four hours before the appearance of the rash, and then for about another week Infection has occasionally been traced to dried crusts It should be emphasised that mild though the disease may be, it is extremely infectious, particularly in the early stages

An isolation period of fourteen days should be ample in the average case Contacts should be kept under observation for three weeks, but there seems little point in imposing quarantine before the tenth day after exposure As in measles, it may be taken for granted that infection will follow exposure in practically every susceptible

One attack will usually confer immunity for life, but this does not seem to be so solid as in the other infections and second attacks have been reported Because of our relative ignorance of the properties of the virus little progress has been made towards achieving any degree of success with active immunization Localized rashes have been produced by the inoculation of vesicle fluid, but this method, apart from the

other obvious objections, suffers from the unpredictability of the results

Passive immunization, even when attempted by the injection of convalescent serum, has been disappointing Gamma globulin is of no value

PROPERTIES OF THE VIRUS

Elementary bodies measuring about 200 mμ occur in the vesicle fluid As already mentioned, they are smaller and far less numerous than in the corresponding variola lesions

Eosinophilic intranuclear inclusion bodies, similar to those of herpes zoster, are found in the epidermal cells of the vesicle Their relationship to the virus of varicella has not been proved but, judging by experience in other diseases, they possibly represent colonies of the virus

The only experimental animals of value are rabbits and monkeys, in both of which the virus has been successfully propagated intratesticularly All attempts at cultivation in the chick embryo have failed

The virus is closely related to that of Herpes Zoster and their affinities are discussed under that heading

Chapter Eleven

HERPES

HERPES ZOSTER
(Shingles)

When clinicians speak of "herpes" they usually refer to herpes zoster Virus workers, on the other hand, reserve the word "herpes" for herpes simplex, and, when speaking of herpes zoster use either the full name or refer to it simply as "zoster" It is in this sense that the words are used throughout this work

Zoster can be an extremely severe and incapacitating disease in adults, on whom the main incidence falls Little is known of the properties of the virus, due principally to the fact that no suitable experimental animal has yet been found in which to study it There is a close relationship between the viruses of herpes zoster and of varicella This has been shown to exist by epidemiological and immunological findings rather than by critical studies of the two viruses themselves

Cases are usually classified as *primary* or *secondary* herpes zoster. The former is "idiopathic" in origin, while the latter accompanies or follows conditions such as spinal tumours or leukæmia The use of the terms "idiopathic" or "essential" is incorrect, as the condition is infectious, even though it may be very difficult to trace the source of the infection From the occurrence of "secondary" cases it is clear that the virus may remain latent for a time, probably in the posterior ganglia, and become active after some stimulus affecting these ganglia has been provided by the other conditions In this latency there is an obvious similarity between it and herpes simplex.

Clinical Features

After an incubation period of seven to fourteen days, an attack begins with pyrexia and frequently with pain along the course of a sensory nerve Three to four days later papules appear along the superficial course of the nerve and these rapidly develop to become the characteristic vesicles Occasionally a few aberrant vesicles may also be seen on other parts of the body The regional lymph glands become enlarged and tender

The pain in the affected area may be very severe, but relief is usually obtained once the vesicles have progressed to the crusting stage This process takes between five and ten days The crusts ultimately separate and a scar may be left In many cases, and particularly in older people, severe neuralgic pain persists for weeks or even months after healing

Zoster is most common on the trunk and is usually uni lateral, but bilateral and even generalized eruptions have been reported The face may also be affected (*cephalic zoster*) by the involvement of the sensory division of the trigeminal If the cornea is attacked the sequelæ may be very serious due to scarring following the corneal ulceration When vesicles occur in the mouth or pharynx they are acutely painful

Paralysis, usually transient but occasionally permanent, may occur Its pathogenesis is unknown but in some cases is probably due to the involvement of the anterior horn cells in a severe attack It is most common following cephalic zoster when as many as 50 per cent of cases may show cranial nerve palsies

Changes may also be found in the cerebrospinal fluid during an attack The globulin level is raised, and the lymphocytes are moderately increased in number Meningo encephalitis is a rare occurrence

Treatment

Treatment is mainly directed towards the relief of pain which may be difficult to achieve, and towards the prevention of secondary infection in the vesicles

Aureomycin and chloromycetin have been reported to be of considerable value and to have had a dramatic effect in some cases On the other hand the condition has occurred in one patient who was actually undergoing treatment with aureomycin for another condition when the eruption appeared

Pathology

Inflammatory changes are found in those dorsal ganglia which serve the area involved in zoster of the trunk and in cephalic zoster similar changes are seen in the extramedullary ganglia of the cranial nerves Microscopically there is marked congestion and infiltration with lymphocytes, inflammation

There is also some evidence that the pathological changes are not exclusively confined to the affected ganglion as there may also be a localized myelitis and leptomeningitis

In the skin the vesicles are confined to the malpighian layer, but there may also be inflammatory changes in the corium Eosinophilic intranuclear inclusion bodies similar to those found in herpes simplex are found in the affected cells Very little experimental work has been done on these inclusions but on an analogy with the other infections in which similar structures are found, they probably represent virus particles

Epidemiology

Cases of zoster are known to occur in contacts either of zoster or of varicella, but the method of spread is uncertain It seems likely to be by means of infected droplets

Similarly it is doubtful how the virus enters the body, or how, once there it reaches the posterior ganglia It most probably enters the body by means of the pharynx or tonsils The suggestion has also been made that the virus enters through the skin and progresses along the perineural lymphatics to the affected ganglion

Hardly any work has been done on either of these problems and while the statement is made that infection takes place by droplet spread it should be understood that there is very little real evidence for it, such as can be given in the case of other infections Neither the portal of entry, nor the mechanism whereby the virus is excreted in the nasopharyngeal secretions, has been demonstrated with any degree of certainty—except in those cases in which vesicles occur in the mouth and pharynx, or which arise from contact with a case of varicella The importance, or otherwise, of skin contact requires evaluation

It has also been suggested that carriers may play a part in the spread of infection

One attack of zoster almost invariably confers a permanent immunity, second attacks being a rarity There is a widespread impression that infection with zoster confers protection against varicella and *vice versa*, but this is not fully borne out by epidemiological observations

RELATIONSHIP BETWEEN ZOSTER AND VARICELLA

The relationship was first postulated on clinical grounds towards the end of the last century and has been repeatedly confirmed since then While neither virus has been extensively investigated, experimental work also points to a close relationship between them, as is shown by the similarity of their inclusions and, more important, by serological cross reactions

It would appear that zoster is more likely to give rise to varicella in contacts than is varicella to induce zoster but it may not be out of place to remark that varicella gives rise to secondary cases of varicella far more frequently than it does to cases of zoster

In a consideration of the relationship of the two viruses it must be kept in mind that zoster occurs most commonly in the adult, and varicella most commonly in the child This

has led to the theory that the two diseases are caused by the one virus, which generalizes in the non immune contact and causes a clinical attack of varicella, while an attack of zoster represents the same infection in a person partially immune to the virus These are most interesting speculations but at the moment they remain speculations The two viruses are unquestionably closely related but there is, as yet, no proof that they are identical

Properties of the Virus

Elementary bodies of about 250 mμ in size have been described in vesicle fluid, but it has not yet been shown with certainty that they represent virus particles

Similarly there is no agreement that any experimental animals are susceptible The virus will survive for a time in tissue culture, and it will proliferate in human skin grafted on to the chorio allantois of the chick embryo It will not grow on the membrane itself nor in any of the cavities of the fertile egg

HERPES
(Herpes simplex, Herpes febrilis)

In dealing with virus infections we have become accustomed not unnaturally, to think of the host virus relationship almost entirely in terms of the host When a man or an animal is attacked by a virus and they show clinical signs of infection we tend to concentrate on their efforts at survival We may forget that the same incident of infection is an expression of the parasite's—that is the virus'—attempt at survival Viewed from a wide biological aspect the most efficient viruses are those which either have a multiplicity of available hosts and an easy method of transfer from one to another, or which, once established, can live quietly for long periods causing the minimum amount of inconvenience to the host

Herpes virus is one of the latter group, and owing to its high degree of parasitism it is one of the most interesting of

the many viruses which infect man We now have a reasonably clear idea of its method of first attack and its efforts, once it has been implanted in the tissues of any individual, to remain there for the lifetime of the host Its study opens up many avenues of speculation on the general principles of the inter action of host and virus, and many of the theories regarding latent infection with viruses spring from such studies It should be added that we still do not know how many of the conclusions reached regarding herpes are applicable to other diseases Nor, indeed, are all workers satisfied that the present theories represent the complete picture of the events which follow infection with the virus Nevertheless, while there are still some gaps a coherent story can now be presented which is probably correct in its main outline

Primary infection most often occurs in childhood, between the ages of six months and six years, and is marked by a more or less severe systemic illness Before the age of six months the infant is almost always protected by maternal antibodies Once the virus has entered the tissues it will remain there for life Even if infection be delayed until adult life the picture is the same—a severe primary attack and then continuing infection

It would seem that social differences may modify the epi demiological pattern to some extent Antibody tests indicate that in the lower income groups infection is almost universal while the more wealthy tend to have a lower incidence The most important single factor concerned in this difference of distribution is probably overcrowding, which makes trans mission easy, and the other advantages which the well to do may enjoy, such as better nutrition play little part

CLINICAL VARIETIES

When it first is encountered herpes virus gives rise to a *systemic illness which may be severe and even fatal* Later recurrent attacks may be unpleasant for cosmetic or other reasons, but they do not give rise to a systemic reaction

As already mentioned the primary attack most commonly occurs in young children In these it causes the clinical condition known variously as aphthous stomatitis, gingivo stomatitis, or ulcerative stomatitis It is now recognized that these conditions are caused by first infection with the virus of herpes

Primary Herpes (Aphthous Stomatitis)

After an incubation period of three to five days the onset is abrupt with pyrexia which may range as high as 104°F The gums are red and tender, and a varying number of vesicles

[illegible]

are enlarged and tender The tonsils are occasionally affected and a diagnosis of tonsillitis may be made in some cases The attack takes about ten days to a fortnight to subside

Such an illness may also occur in adults if they have not been infected in childhood In both children and adults secondary bacterial infection is common Sulphonamides and penicillin may help in combating the latter but have no effect on the primary virus infection

On epidemiological grounds there seems little doubt that sub clinical primary infection, without the severe symptoms described above, must be relatively common and that the primary attack may pass unnoticed On the other hand outbreaks involving whole families have also been reported

Recurrent Herpes

In these cases the virus, which persists for life probably in epithelial cells in the mouth erupts from time to time These eruptions long after primary infection are, however, always localized and no systemic attack occurs In some instances the virus may be found in the saliva even between attacks It becomes active again apparently in response to some stimulus which is as yet, unknown An alternative

reason for the renewed activity may be a periodic susceptibility of the host's cells For example, recurrences often occur in women during menstruation It is also possible that the strains of the virus involved in these cases have somewhat unusual properties

The varying conditions known as herpes simplex, febrilis, cornealis and genitalis, are all manifestations of herpetic infection

Herpes Febrilis

This is common during attacks of pneumonia and influenza when the vesicles are commonly found on the lips

Herpes Cornealis

Ocular infection with herpes virus may manifest itself as a conjunctivitis, kerato conjunctivitis or as dendritic ulcer Any ulceration of the cornea is, of course, dangerous as it may lead to secondary infection and consequent scarring Aureomycin is said to be of value in these cases

Kaposi's Varicelliform Eruption

This rare condition appears to be another manifestation of herpes infection

Involvement of the Nervous System

[illegible] encephalitis

[illegible] s a

rapid course The prognosis is good In meningo encephalitis there is pyrexia altered reflexes and varying degrees of paralysis The diagnosis cannot be made clinically as the symptoms and signs are similar to those of meningo encephalitis due to other viruses It rests therefore on the isolation of herpes virus from the cerebrospinal fluid or, in fatal cases, on the demonstration of the characteristic intra nuclear inclusions which are described below

Laboratory Diagnosis

The virus is present in high concentration in the vesicle fluid in all types of herpes and it can be isolated from it or from infected tissues by the inoculation of various animals and the chick embryo Rabbits are the most widely used experimental animals and they are commonly infected by scarification of the cornea In positive cases this will produce a keratitis any time between a few hours and a week later They may also be inoculated intracerebrally and will then develop encephalitis With chick embryos the chorio allantoic membrane is used, and characteristic pocks are produced on it

Elementary bodies have been described in films prepared from vesicle fluid but they have not been fully identified as virus particles

As infection produces a lasting immunity there will be a significant difference in the antibody level between acute and convalescent sera Virus neutralization or complement fixation tests may be used These serological tests, which depend on a rise in antibody titre, are only available for the diagnosis of primary infection

Histological evidence of infection is obtained by finding the typical intranuclear inclusion bodies in infected cells They are present in epithelial cells, and in fatal cases of meningo encephalitis are also found in the cells of the nervous system

Pathology

The histological features vary, as might be expected, depending on the nature of the tissue involved Typical vesicles may best be seen in sections of skin tissue The lesion is superficial to and does not involve, the corium—hence there is no scar on recovery There is an acute inflammatory reaction around the affected areas

The characteristic feature is the intranuclear inclusion body found in infected cells Known as Lipschutz bodies these are homogeneous irregularly shaped eosinophilic structures which

may occupy most of the nucleus They are found in all cells which are actively infected by the virus—not merely human cells but also in tissue cultures and in the infected chorioallantoic membrane of the chick embryo They almost certainly *are aggregates of virus particles* and not just cellular reaction products A single inclusion body of this nature has produced a typical keratitis in the rabbit

In the brain, perivascular cuffing and petechial hæmorrhages are found in fatal cases of meningo encephalitis, principally in the cerebrum, pons and medulla There is a marked destruction of ganglion cells in the cortex, and a considerable *degree of gliosis is found in the neighbourhood of* these lesions Typical inclusion bodies are found in many of the glial cells

Epidemiology

Infection with herpes virus is widespread in most communities, and few reach adult age without being attacked *Man is the only reservoir and the virus is spread* by direct contact, or possibly by fomites such as cups and spoons contaminated by infected saliva Children are not only peculiarly susceptible, as their mucous membranes appear to be easily infected but they are also probably at greater risk through, for example, being kissed by infected adults It does not seem to be necessary for such an adult to be suffering from a clinical attack at the time

Once introduced into a household the infection will involve all the susceptibles in it with varying degrees of severity Some may escape a clinical attack, and others may be severely ill

No method of controlling the spread of the disease is yet available

As to the survival of the virus, the most popular theory at present suggests that after a primary attack the virus persists in the body as a latent infection which erupts and becomes clinically obvious from time to time The reasons for these later eruptions are *still unknown*

The concept has much to recommend it. It seems difficult to believe that each attack, in those suffering from recurrent herpes, represents a fresh infection. If this did happen in practice one would expect to find either very low or negligible antibody levels, or to find them fluctuating as happens in influenza. It is now apparent that while no antibodies to herpes virus are present before the primary attack, they are present afterwards and, once present, persist for life at a high titre. That this is a constant finding is shown by widespread surveys of antibody levels in different countries. These have revealed that they are either very high, or that no antibody at all can be demonstrated. The constant breakdown of infected cells may act as a stimulus to keep the antibody mechanism in high gear.

Properties of the Virus

Size

The reports of the size of the virus particle vary according to the method used to estimate it. By filtration through collodion membranes it was found to vary between 100 and 150 mμ. Centrifugation studies give a rather higher figure.

Resistance

The virus will survive for about one year in infected tissues when stored in 50 per cent glycerol. It is sensitive to heat, being killed by thirty minutes exposure to 56°C., and is rapidly inactivated by ultraviolet light.

Experimental Animals

Growth occurs in tissue culture and in the chick embryo. In the latter typical pocks are found on the chorio allantoic membrane after inoculation by this route. Rabbits, mice, guinea pigs and a number of other experimental animals are susceptible.

Strain Differences

These have been extensively studied in the rabbit which develops keratitis after corneal scarification, or encephalitis

after intracerebral injection With some strains the animal develops encephalitis after corneal inoculation, human strains isolated from a primary attack being likely to produce an experimental encephalitis These degrees of neurotropism would, however, in general seem to depend more on the host than on the virus There seems no reason to believe that a strain which produces encephalitis in the rabbit is necessarily any more dangerous for man than one which does not

No relationship exists between the viruses of herpes simplex and herpes zoster.

Chapter Twelve

VARIOLA AND VACCINIA

It is a commentary on the haphazard development of the techniques of virus study that vaccinia virus, causing what is a minor infection as distinct from its protective importance, should be still the best defined member of the animal virus group Variola or smallpox, virus on the other hand, which is responsible for so much serious illness is still a relatively unknown entity to the laboratory worker

Smallpox appears to have been present in Asia throughout recorded history, but after its widespread prevalence in Europe in the seventeenth and eighteenth centuries it has now declined to the status of an infrequent, although thoroughly unwelcome, visitor A similar decline has occurred in America Burnet suggests that its unimportance in western Europe America and Australia largely accounts for our relative ignorance of the properties of the virus, as it is in these countries that the laboratory study of viruses has been most advanced Again, it is only in quite recent years that a simple medium—the chick embryo—for the growth of variola has been discovered, and that the opportunity has arisen for its detailed study

Vaccinia is linked with the name of Edward Jenner, it is of importance because it protects against variola This, one of the few established facts in medicine, is still, more than 150 years after its discovery, the focus of bitter controversy. In few places is vaccination now compulsory, with the result that those responsible for the public health have to be constantly on their guard against outbreaks of a preventable disease After a century and a half the battle has been lost and those countries which have abandoned compulsory

vaccination increase in number almost yearly It is, perhaps, an inverted compliment to public health authorities, but one which they would readily forego!

VARIOLA

Two varieties of smallpox occur—*variola major*, or classical Asiatic smallpox, and a milder form, known variously as *Alastrim*, *Kaffir Pox* or *variola minor* It seems preferable to use the term *variola minor*, because it serves as a reminder to workers in the field that they are, in fact, dealing with smallpox and not with a distantly related variant It is this form which was prevalent in England and the United States during the 1920's Usually mild it may cause serious sequelæ, disfigurement and even death

Variola Major

The incubation period is twelve to fourteen days

The onset is sudden with pyrexia, headache, pains in the back and legs, vomiting and abdominal pain In this stage the condition resembles influenza but respiratory signs are not marked On the second day prodromal rashes appear in some ten per cent of cases These may be erythematous in type resembling the rash of measles or of scarlet fever and are distributed through the body Another variety of the rash—petechial—has a local distribution, being usually confined to the axillary and lower abdominal areas In the latter it is characteristically the 'bathing drawers' rash Petechial *prodromal rashes usually herald a bad prognosis*

In these early stages the patient is obviously ill, toxic and drowsy and may become delirious Albuminuria is a constant finding

On the third day the temperature falls the clinical appearance of the patient improves considerably and the specific rash appears *This shows initially as macules* which are usually first seen on the forehead and are found in the facial region in practically every case Within a matter of hours

they have become papules which are hard and shotty to touch Forty eight hours later the vesicular stage is well developed The vesicles become umbilicated and their contents increasingly turbid, for a period of three to four days from their appearance, and they finally become pustules The temperature rises again with secondary fever due to sepsis from the pustules which are very painful The face and extremities, on which they are most marked, are swollen and œdematous Laryngitis and bronchitis are very frequently present When the pustules rupture the whole area involved is covered with pus and the patient is extremely distressed

About the ninth day of the illness crusting begins, the pustular contents gradually becoming absorbed and replaced by crusts or scabs As convalescence proceeds these separate and leave discoloured areas which slowly disappear The separation process takes about a month In those parts in which the pocks were deep seated the scarring, characteristic of the disease, remains for life

In *hæmorrhagic* or *malignant* smallpox death often occurs before the specific eruption appears Initially the patient is very ill and the prodromal rash prominent Small, dark coloured hæmorrhages into the skin occur all over the body accompanied by severe bleeding from the mouth and nose and into the bowel There is a leucocytosis and a rapidly progressive thrombocytopenia Immature white cells may be found in the circulating blood Death is the invariable outcome

Confluent smallpox shows the typical features already described but as its name implies, the lesions are extremely numerous, particularly on the face, and in the pustular stage whole areas of the body are bathed in pus The patient is appalling to see, obviously very toxic and, until crusting gives relief, in very severe pain

The distribution of the specific rash in smallpox is characteristically *centrifugal* When it is fully matured the pocks are found in most profusion on the face and are more marked on the forearms, wrists, and hands than on the upper arms

The same pattern is found on the legs On the trunk the lesions, less numerous than on the limbs, are thicker on the chest than on the abdomen, and more numerous in the scapular region than the lumbar It should be emphasized that the eruption does occur on the trunk Pocks are also found in the mouth and on the fauces They do not occur in the flexures or in the axillæ Modification of this distribution may be caused by previous trauma or by pressure due to clothing In such areas the lesions are profuse

The time taken by the eruption to reach any given phase is relatively constant, and all the lesions are at much the same stage of development at any one time, which is an important point in the differential diagnosis On the other hand, the rash may not be fully out until four or five days from the beginning of the illness This means that the significance of the distribution may not be fully appreciated for some days, and this delay may be very dangerous in the early stages of an epidemic The modification of the rash by previous vaccination will be referred to later

Variola Minor

The incubation period is ten to fifteen days, and an attack starts with a sudden high temperature with headache, back ache, pain in the limbs rigors nausea and vomiting The patient is not as ill as in the corresponding stage of variola major

The time of the appearance of the rash is more variable than in variola major Cases have been reported in which it came out as early as the first day or as late as the tenth In the great majority of cases it appears on the third or fourth day being, as a rule, scantier than in variola major The lesions appear first on the face but within a matter of hours are found on the arms and legs Progression to the pustular stage is more rapid than in the major type Papules are present for some forty eight hours or less, and with vesiculation occupying only a day, pustulation is fully developed by

the fifth or sixth day Secondary fever is uncommon Crusting begins on the face by the seventh or eighth day and the crusts have separated in three or four weeks

It is frequently stated that in variola minor the rash appears in crops This is not correct, the misconception being due to its rapid evolution by which, at any given time, the eruption on the face is further developed than it is on the extremities

Death is infrequent and when it occurs is usually due to bronchopneumonia Scarring as a sequel, is not often prominent in variola minor

Varioloid

This term is used to describe mild or modified cases occurring in those previously vaccinated Such cases, especially if mild are of great epidemiological importance They are infectious and are capable of transmitting the fully virulent disease All varieties of smallpox may be encountered in these cases *depending on the length of time since vaccination was performed*

In those who are partially immune as a result of successful vaccination some years before, there are certain factors which will influence the clinical picture If these are understood many of the apparent contradictions in the behaviour of the disease can be explained

Vaccination modifies the course of the disease in three particulars First there is a neutralizing effect on the toxic phase secondly, the number of skin lesions is diminished, and thirdly their progression from macule to pustule is hastened As time elapses after vaccination these gradually lose their effect in the order given, so that in a partially immune patient there may be a severe toxic phase which is followed by a mild and scanty rash A patient in whom the immunity has waned to this extent may even die in the early toxic period In others the skin eruption appears and goes through all its phases rapidly or it may stop at the vesicular stage or even earlier and go no further

Such cases are often missed during an epidemic and, owing to their infectivity, probably explain many of the lacunæ in the search for contact with a previous case

Treatment

There is no specific treatment During the toxic stage a fluid diet may be given and later, including the period of secondary fever, the diet should be kept as full as possible Sulphonamides and penicillin may be of value in controlling secondary infection during the pustular stage

Clinical Diagnosis

In the prodromal stage the clinical picture may resemble influenza, but in some cases the generalized prodromal rash and in all the subsequent course of the disease, outrule it Measles and scarlet fever may also have to be considered for a short while

The clinical differentiation of variola from chicken pox is frequently difficult In the presence of an epidemic of either disease little trouble may arise, but a case of smallpox early in an outbreak could be missed unless the closest attention is paid to the examination

*In all cases the patient should be stripped and the distri*bution of the rash carefully inspected The major points of difference between the two diseases are the distribution and the rate of maturation of the respective eruptions In chicken pox it is most marked on the trunk, and the extremities are usually spared, in addition, successive crops come out so that all stages from papules to pustules may be found in any one small area at the same time

It should be unnecessary to stress that these are only general rules, and that each case must be judged on its merits Many practitioners rarely see a case of smallpox, and should, *if in* doubt, consult their medical officer of health Chicken pox is frequently made notifiable during an outbreak of smallpox All such cases should be seen by the medical

officer of health, or someone conversant with the disease; otherwise the precaution may be nullified

Pathology

Death, when it occurs, usually does so between the tenth and the thirteenth day

In the skin the first change is a degeneration of the cells in the lower levels of the epidermis Gradually a serous exudate appears which is loculated by strands of degenerated cells—the vesicle As the disease continues and the vesicular contents become pustular these strands are destroyed and the fully developed pustule is formed These lesions may occur within the epidermis and be separated from the corium by a layer of intact cells, or they may be deeper and involve the corium The pathogenesis of the subsequent scarring is therefore readily seen It depends on whether or not the papillæ are damaged

Similar changes are found in the mucous membrane of the mouth and œsophagus

Elementary bodies are found in the vesicle fluid, their profusion being an important diagnostic sign, they are difficult to demonstrate in the pustular stage

Acidophilic inclusions—Guarnieri bodies—are present in the cytoplasm of intact cells These are large, varying in size up to 10 μ, and by modern techniques have been shown to be masses of elementary bodies embedded in a matrix They are found in all infections caused by variola or vaccinia viruses—whether in man animal or chick embryo

At post mortem an almost invariable finding is an associated bronchitis and bronchopneumonia No specific changes in other organs are found, but all will show cloudy swelling, and most will show petechial hæmorrhages

Laboratory Diagnosis

In recent years the laboratory diagnosis of variola has been greatly developed, particularly by Downie, MacCallum and by van Rooyen and Illingworth The tests now used are

precise and a result can be obtained rapidly Importations of the disease into Western European countries are relatively frequent, and the possibility of its spread through non immune populations makes it necessary that practitioners, and particularly medical officers of health, should have a knowledge of the tests available They should also be aware of the accuracy of present day methods In at least one outbreak of recent years a positive result from a laboratory was discounted with unfortunate results

Maculopapular Stage

Laboratory tests can be of use as early as the second or third day Scrapings may be taken for microscopical examination from the maculopapular eruption or even the prodromal rash The lesions to be examined are cleaned with spirit or ether, the superficial epithelium removed, and the base of the lesion scraped with a sharp scalpel These scrapings are carefully spread on a small area in the centre of clean glass microscopical slides They are then allowed to dry in air and sent to the laboratory They should be handled carefully as they may contain living virus There they may be stained with either Paschen's or Gutstein's stain and examined for elementary bodies In a case of variola or vaccinia numbers of them are seen, they are not seen in varicella By this method a positive result may be obtained within an hour of the specimen reaching the laboratory These scrapings must be carefully taken and at least six of them sent for examination A negative result on stained films does not necessarily exclude smallpox Also, even if specimens are sent in the early stages, vesicular material must always be forwarded later when it becomes available

Scrapings may also be used for isolation of the virus by egg inoculation This is slower but in the final analysis is the most satisfactory and the most definite method of making a diagnosis at any stage of the infection Downie and Dumbell have used the method with completely satisfactory results, being able to give a definite result two or three days after

inoculation This is a longer time than is required for serological tests but the appearance of variola and vaccinia on the chorio allantoic membrane of the egg are quite distinct Chicken pox produces no lesions on the chorio allantois A possible disadvantage is that a supply of fertile eggs at the proper stage of development must be available Virus has also been recovered from the blood before the onset of the rash so that a specimen of blood should also be sent in a dry tube

It seems very probable that the marked clinical improvement about the time the rash comes out is due to the appearance of neutralizing antibodies in the blood The viraemia is therefore of short duration depending on the time lag before antibodies appear As might be expected these will not influence the course of the rash because the virus is already present in the skin cells and therefore beyond their reach

Virus has been demonstrated most easily in the blood of acutely ill patients the majority of whom subsequently died This could be expected as it is in these patients that the antibody response is poorest

Paul s test—the application of scrapings to a rabbit s cornea after scarification—may also be employed It may give quicker results than the egg inoculation methods but has proved unsatisfactory in the hands of several workers particularly in cases of variola minor and is now little used when fertile eggs are available Serological tests may also be carried out on these early skin scrapings the material being used as the antigen in a complement fixation test

Vesicular Stage

When the vesicles appear scrapings may be taken from the base of a number of them—never less than six—and sent for examination In addition capillary tubes should be filled with the exudate from several large vesicles and sealed If these tubes are not available the fluid may be absorbed on to a sterile throat swab This fluid may then be used for isolation complement fixation or flocculation tests While

neither of the latter will differentiate between variola and vaccinia, important if the patient has been recently vaccinated, the complement fixation test is extremely delicate, and a diagnosis of variola or vaccinia is usually all that is required Such a report may be given within twenty four hours

Pustular Stage

Any of the above methods of examination may be used except the microscopical, as elementary bodies are then very difficult to distinguish The pustular exudate may be collected in a sterile tube or, better, in a sterile screw capped bottle

Crusts and scabs may be sent for complement fixation or isolation tests in a sterile tube or bottle

After about the eighth day blood may also be sent for the detection of antibodies in the patient's serum Not less than 5 ml should be forwarded in a dry tube This test, as it can only be performed late in the disease, may be regarded as a confirmatory one, but is valuable if the patient has not been vaccinated within the previous six months

The complement fixation test is that most widely used but anti-hæmagglutinins, which are at their peak about the tenth day, can also be demonstrated Either test is more valuable if an earlier specimen of blood, taken within the first four days of illness, is also available , a rise in antibody level can then be demonstrated in positive cases

In many cases the clinical findings enable a confident diagnosis to be made, but no opportunity should be lost of obtaining laboratory confirmation None of the tests are of a routine nature, and every public health authority should have prior arrangements made with a laboratory capable of undertaking them in case the need arises

Epidemiology

The disease is widespread throughout the world In Great Britain and the United States variola minor was relatively

common and indeed prevalent until the early 1930's. Variola major is rare, but importations of it may occur at any time, and did in fact occur into Britain in 1942, 1944, 1946, 1949 and 1950. In Great Britain outbreaks of variola major have been usually traced to a case occasionally not diagnosed in time occurring on a ship returning from the Far East and infecting the passengers or crew who have landed and dispersed. In the 1950 outbreak in Glasgow the cases occurred amongst the contacts of a seaman who fell ill after he had left the ship.

It is also possible for a person to travel a long distance by air during the incubation period as happened in the Brighton outbreak. Those previously vaccinated who develop modified smallpox may be very dangerous, as the disease is modified only for them and they are capable of transmitting it in a fully virulent form to others.

Spread

The virus enters the body by the respiratory tract but the sequence of events thereafter is not by any means clear. If Fenner's analogy with mousepox can be accepted the virus may multiply in a primary focus either in the lung tissue itself or in the regional lymph glands. Thence it spreads throughout the body by the blood stream and continues to multiply probably in the spleen and liver and possibly in the lungs. At the end of the incubation period the secondary viraemia occurs leading to infection of the skin and mucous membranes and to the specific eruption.

Little experimental evidence however has been put forward for this viewpoint and until the existence of a primary focus of multiplication in variola has been demonstrated it must remain somewhat speculative.

There is some evidence that a small proportion of cases may be very infectious before the onset of the specific rash which would suggest that the lung is important as a primary site of multiplication. In such cases a focus of multiplying virus in the lung may have ruptured into a bronchus. This

would allow of massive dissemination of virus to the environment, as against the more usual liberation of virus from the lesions in the mouth and pharynx following the secondary viræmia

Virus is present in the skin lesions from the time the rash appears but there is no reason to suppose that it is liberated until necrosis begins, that is until the pustules erupt Once that happens a considerable amount of virus is, of course, present in the immediate neighbourhood of cases The skin is then infectious until the last crust has been shed and all the lesions have completely healed Infection can be transmitted *by fomites, and all bed clothes, crockery and other utensils* in contact with the patient must be carefully sterilized This precaution must be taken for many weeks until the patient is non infectious The risk to those in hospital laundries which are dealing with the bed clothes of patients is very considerable, and all such clothes should be disinfected before being sent to the laundry Similar considerations arise with *persons coming into contact with the bodies of those dying* of smallpox, for example in laying them out

The virus may survive in the dry crusts for more than a year, and has also been isolated from the dust near the bed of a case

There has been acute controversy for many years on the distance which infection can travel through the air Previously it was the accepted view that such a means of spread was of *great importance*, and smallpox hospitals were therefore invariably located at some distance from any centre of population By 1942 the pendulum had swung in the opposite direction, and in the Edinburgh outbreak of that year cases were nursed in a separate pavilion in the Fever Hospital without any ill effects It is indeed quite possible that cases of smallpox could be safely nursed in an isolation one bed ward of a fever hospital

Irrespective of these speculations however the most rigid precautions must be enforced in any hospital with smallpox patients All contacts such as doctors, nurses and domestic staff must have a *high level of immunity from frequent*

re vaccination, separate laundry facilities have to be provided, and ambulance drivers, and even tradesmen, must be fully protected. The risk of airborne spread except in the immediate vicinity of cases, is probably negligible but the risk of spread by the mild or modified disease in those who are only partially immune is very real. Together with the prolonged viability of the virus in crusts and scabs it constitutes the fundamental difficulty in smallpox control. It probably accounts for the so-called "airborne spread" over considerable distances described by the early epidemiologists.

Control

Isolation

All cases must be isolated until no longer infectious.

Contacts

Once a case has been reported a comprehensive list of all contacts should be drawn up—including, as far as possible, all those who may have been in even casual contact with the patient. It will obviously be impossible to trace all of these but it may be feasible to warn the general population that a case has occurred and to give a plan of the patient's movements in public places, for example, cinemas. It should be made clear that those who were in such places at the same time might have been exposed to infection.

The list of more intimate contacts such as those living in the same house and visitors to it, should be very closely supervised. They should all be offered vaccination—it is unlikely that many will refuse it. Daily visits for three weeks should be made and any illness immediately investigated. More particularly the closest watch should be kept on those who have been vaccinated as in them the disease may be modified by the vaccination. In some areas it may be advisable to isolate intimate contacts in special isolation hospitals and there should be no hesitation in doing so should it be considered necessary.

Mass Vaccination

Vaccination is the shield against smallpox and the surest safeguard against its occurrence is a fully vaccinated population During an epidemic mass vaccination of the population must be considered and it has been carried out in many cases in recent times *The decision to undertake it should be weighed very carefully* While no considerations of administrative difficulties or of alarming the public should be allowed undue weight, the risks of post vaccinal encephalitis and other complications must be taken into account It is very difficult to forecast how smallpox will behave in any population and the guiding rule may be taken as the occurrence of cases without any history of contact When an epidemic begins to spread in such a fashion mass vaccination must be seriously considered

During the 1946 epidemic in Tripolitania Dixon acted on *the principle of raising a barrier of immunes around* the primary case by the selective vaccination of close contacts This expanding ring method would seem to have much to recommend it and was eminently successful in his hands It is of course a modification of the method used in hospitals by which the patient is surrounded by a ring of vaccinated attendants

Ships and Aircraft

These are covered by International Regulations which prescribe for the vaccination and the isolation or surveillance of contacts on board ship and the disinfection of those parts of the vessel occupied by patients The period of observation may not exceed fourteen days

Air travel has its special difficulties and persons who have *left an epidemic smallpox area and* who are not sufficiently immunized may be vaccinated on landing and be subjected to observation for fourteen days In this connection observation may mean isolation or surveillance Should a case *actually occur* on a plane the steps to be taken are similar to those in effect for ships

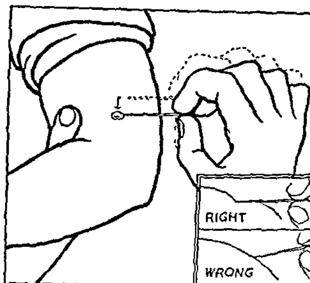
VACCINATION

Immunization against smallpox by the deliberate production of a mild attack—variolation—goes back many hundreds of years. It was first introduced into England in 1717 and was made illegal in 1840 but after Jenner's report in 1798 of the immunity offered by cowpox inoculation it had been generally superseded by vaccination. The circumstances of Jenner's discovery are too well known to need recapitulation, but at a time when vaccination is no longer compulsory in England or Ireland, it should be realized that within the past one hundred and fifty years it has saved many thousands of lives.

Jenner's original material for vaccination was derived from a cowpox lesion and cowpox was for many years regarded as identical with vaccinia. Recent work has shown that this is not so but nevertheless after artificial inoculation it is capable of protecting against variola.

Vaccine lymph, the material used nowadays for vaccination, is prepared by inoculating calves or sheep with vaccinia virus, which has been produced in the laboratory by the repeated passage of either variola or cowpox virus in susceptible animals. The precise origin of the 'seed' virus used in different countries varies in its derivation from either variola or cowpox strains. Such differences of origin are of little practical significance when considering its protective powers. Vaccinia virus loses its virulence and therefore the protection it affords with constant passage in the same animal and it is necessary to pass it occasionally through a different animal, usually the rabbit, to exalt its virulence before it is again inoculated into calves. By this means fully active vaccine lymph is kept constantly available. The production of the lymph is regulated by the Therapeutic Substances Act, which lays down standards of potency and purity. Most lymph has been kept in cold storage for about two months before use. When its bacterial count has fallen to five organisms per milligram or less the lymph is fit for issue—

and *should not draw blood*, the skin being stretch than scratched The side of the needle point causes trauma for a successful "take"



Intradermal

The advantages claimed for this method are the av *of secondary infection and minimal scarring* It regarded as being still in the experimental stage, and be recommended for general use at present

A light non antiseptic dressing may be applied aft cination although many do not regard it as necessa vesiculation occurs An antiseptic dusting powder c cillin powder may also be used once the vesicle has ap The area must be kept dry until the crust has separ

If the technique is adequate primary vaccination should be successful, and it should rarely be necessary to repeat it because of a lack of response. Occasionally, individuals are met with who show no reaction even with potent lymph and proper technique. In the great majority of these cases some type of reaction can be observed if carefully looked for. If, after three attempts on different areas of skin with different batches of lymph, there is no evidence of any reaction such cases may be regarded as insusceptible to vaccinia. This does not necessarily mean that they are insusceptible to variola, although probably most of them are.

Primary vaccination is best performed in infancy preferably between four and six months of age. At this time reactions are less severe and post vaccinal encephalitis, never common, is extremely rare. Revaccination may be done after five years and again about fifteen years later, or during an epidemic. Those specially exposed to risk should be revaccinated yearly.

Clinical Course

It should be appreciated that successful vaccination is due to infection with vaccinia virus and at one stage of the infection the virus is widely distributed throughout the body.

On the third day a papule appears at the site of primary vaccination. By the fifth day this has become vesicular with a surrounding areola. It gradually increases in size, the contents becoming more turbid until the eighth day. At this stage there may be marked constitutional disturbances with pyrexia and lymphadenitis of the glands draining the site of vaccination. The lesion becomes definitely pustular about the ninth day. After the twelfth day the pustule begins to dry and a scab forms which separates in another two or three weeks. The resulting scar is usually visible for life.

In a small proportion of cases a non specific erythematous rash appears between the seventh and the fourteenth day after vaccination. Generalized vaccinia, characterized by a widespread skin infection, is a very rare complication which

occurs only once in every 100,000 cases It is more likely to occur in children suffering from skin affections Together with active tuberculosis these should be regarded as contra indications to vaccination

Revaccination

In re vaccination any one of three reactions may be encountered

If a considerable time has elapsed since primary vaccination a typical primary response will probably be obtained

In those with some residual immunity an accelerated or vaccinoid reaction, with vesiculation, occurs reaching its height between the third and seventh day Constitutional reactions, if they occur at all, are mild

In some cases a papule appears within twenty four hours of vaccination but does not develop into a vesicle This was formerly regarded as an immune response, but it is now realized to be merely an allergic reaction and of no value in raising immunity to smallpox The proof of developing immunity may be taken as the appearance of a vesicle Vaccination should be repeated in such cases

For the purposes of certification the patient should ideally be inspected on the second, fourth and seventh days Occasionally this is not possible, and *the inspection should* be carried out on the seventh day in the case of a primary vaccination, and on both the third and seventh days where revaccination has been performed The International Certificate recognizes three reactions viz primary, accelerated or vaccinoid and "reaction of immunity," and the appropriate term should be used A statement of "no reaction" will not be accepted

Duration of Immunity

By the time the vesicle has formed the patient is *generally* resistant to smallpox, and immunity is certainly conferred by the tenth day following primary vaccination Its duration is variable and no general rule will cover all cases, but it is *generally agreed to last between* five and ten years *If there*

be any serious danger of contracting smallpox, for example in the case of doctors and nurses or in those living in endemic areas, revaccination should be performed at frequent intervals, usually yearly That protection against smallpox is afforded by vaccination, provided the immunity is kept up by re-vaccination, is too well known to need further mention, but the vaccination must be done before exposure to smallpox or immediately afterwards Generally speaking if vaccination is performed within three days of exposure it will probably be protective, but there are exceptions to this rule It will almost certainly modify the attack but may fail to prevent it in all cases

POST-VACCINAL ENCEPHALITIS

The ætiology of this rare complication is still unknown Ten to thirteen days after vaccination the patient suddenly becomes acutely ill with headache, squint pyrexia, vomiting and signs of meningeal irritation Spastic paralysis is frequently present There is a marked leucocytosis in the blood, and an increase in the cell content of the cerebrospinal fluid The mortality is between thirty and fifty per cent When recovery occurs it is usually complete

Pathology

Histologically there is a characteristic perivascular demyelination, perivascular cuffing with lymphocytes and, in some cases, hyaline thrombi in the vessels These changes are widespread and are found in the cortex pons and medulla In the cord there is a well marked demyelination of the white matter

Ætiology

No agreement has been reached as to the ætiology of the condition A similar encephalitis follows several other virus infections notably measles, varicella and anti rabies inoculation, so that vaccinia virus itself does not seem to be the cause.

A superficially attractive suggestion is the activation of a latent virus in the central nervous system, but there is no proof of this Alternatively the condition may be allergic in origin It has also been suggested that the patchy areas of demyelination are due to multiple small cerebral thrombi The one thing common to all cases is a virus infection, which seems to be the precipitating cause, but as far as vaccinia is concerned no particular batches of lymph, or strains of vaccinia virus, have ever been implicated

Whatever its origin the condition is a rare but definite complication involved in infections, deliberate or otherwise, with a number of viruses Their characteristics and clinical features are so varied that it is difficult to see why they should have this rare sequel in common No latent virus such as has been mentioned has yet been isolated from such cases, possibly because of defective technique or equally possibly because it is not there In other words the ætiology of the condition remains a mystery

Incidence

Encephalitis is very rare after primary vaccination in infants The main incidence is in older children particularly those vaccinated for the first time between six and fourteen years of age It is also very rare after revaccination

Nowadays it is uncommon and seems to be associated with mass vaccination campaigns but there were many cases in Holland and in England during the 1920's It is rare in the United States of America

CHARACTERISTICS OF THE VIRUS

Variola

ELEMENTARY BODIES

As already mentioned numerous elementary bodies, measuring some 250 mμ in diameter, are found in the lesions in cases of smallpox These are termed Paschen bodies, although they were first described by Buist of Edinburgh *On an*

analogy with the findings in experimental work on vaccinia virus it is now believed that these elementary bodies are virus particles They are readily stained by Paschen's or Gutstein s methods

CHEMICAL OR PHYSICAL AGENTS

As is shown from the discussion on epidemiology, the virus is exceptionally resistant to physical and chemical agents It will survive for years if stored at a low temperature or if dried by even the simplest methods and is resistant to 50 per cent glycerol Downie and Dumbell have shown that desquamated crusts from cases of smallpox still contained living virus after a year's storage in a stoppered bottle which was kept in the dark

Vaccinia

The relationship between the two viruses is obviously very close Perhaps the one distinction between them is that one, variola occurs in nature, and is a member of the large group of viruses which affect particularly domestic or domesticated animals Vaccinia virus on the other hand is a laboratory product derived by animal passage from cowpox or variola, and only infects man by inoculation or by direct skin contact Being easy to handle the volume of work done on it is enormous and completely out of proportion to its epidemiological as distinct from its biological importance

ELEMENTARY BODIES

As in variola these are easily found in the lesions using the same technique Their diameter is about 225 mμ Statistical studies point overwhelmingly to the conclusion that under proper conditions a single elementary body can initiate infection It may be taken therefore that the elementary body is the virus particle

RESISTANCE TO PHYSICAL AGENTS

Like variola vaccinia virus can be readily stored in an active condition at low temperature or in 50 per cent glycerol

Desiccated *in vacuo* it survives for years It is very rapidly destroyed by the photodynamic action of methylene blue

HÆMAGGLUTINATION

Vaccinia virus suspensions will agglutinate chick red cells in a manner analogous to the influenza group In this case, however, the agglutination is not caused by the virus particle itself, as in influenza, but by a "soluble substance" which can be separated from the elementary bodies in the suspensions No elution of the hæmagglutinin occurs.

CHAPTER THIRTEEN

HEPATITIS

THE history of virus hepatitis has been marked by confusion Only recently has it been recognized that *catarrhal jaundice, epidemic jaundice, hepatitis epidemica,* and *infectious* or *infective hepatitis* may be regarded as different names for the same condition As early as the 1830's Bright and Addison, and later Stokes of Dublin, recognized that jaundice could be due to hepatitis, but progress was then retarded for more than fifty years by Virchow's concept of "*catarrhal jaundice,*" said to be caused by a mucus plug in the ampulla of Vater Gradually, as outbreaks of infectious jaundice were reported more frequently and leptospiral jaundice was differentiated, a new outlook on the problem was adopted Nevertheless, even then progress was slow until epidemic jaundice became a major military problem amongst all the armies which fought in the Mediterranean theatre in World War II

At least two viruses are concerned in the production of jaundice in the human, but except for their action on the liver they bear little relationship One is transmitted naturally, by personal contact or fæcal spread while the other is trans mitted artificially by the inoculation of human blood, or its products containing the virus

The best and most widely used names for the respective conditions appear to be *infective hepatitis* and *serum hepatitis*

Infective hepatitis is an infectious disease with a long incubation period It is most common in children, and is transmitted by fæces or infected droplets An attack gives rise to a diffuse inflammation of the liver parenchyma with the production of variable signs and symptoms, of which the most constant is jaundice The prognosis in the a

phase is excellent, as the mortality is low, but not enoug
yet known of the long term results following clinical recov

CLINICAL FEATURES

Incubation Period

This is between twenty and forty days, although during epidemic the time may be shorter in some cases In gene judging by the findings in epidemics, adults tend to hav longer incubation period than do children Many experime have been carried out with human volunteers, and amon them the average incubation period has been about twen five days

The clinical features of infective hepatitis are similar adults and children, except that the condition is usua more severe in the adult

Adults

In the typical case two stages can be distinguished clinica —a pre icteric phase and one of frank jaundice A few ca may be jaundiced from the beginning of their attack

The onset is insidious with anorexia as a prominent sig and it is followed by malaise, headache and abdominal d comfort Pyrexia ranging as high as 102°F may occur b is not invariable, tachyardia is the rule at this stage Re piratory signs are usually absent Physical examinati reveals little, but enlarged glands may be found in t posterior triangle of the neck Towards the end of this stag the liver is palpable and tender Histological eviden obtained by biopsy shows that hepatitis is already present

After five to seven days the temperature falls to norma and a day or so later jaundice appears This varies in degr from an evanescent icterus of the conjunctivæ to a dee yellow coloration lasting three to four weeks *In sever* cases it may persist for months The temperature remain normal during the jaundiced phase In mild cases the anorexia

malaise and headache usually disappear with the advent of jaundice, but in a severe case they remain prominent for another ten to fourteen days Once the height of the jaundice is passed a sense of well being returns, there is rapid relief from symptoms and an increase in appetite

During the course of an attack there is often a distinct loss of weight, which may be up to 10 lbs Pruritus occurs in a minority of cases, but it lessens as the jaundice clears Similarly the liver gradually diminishes in size and within [illegible]

Three months is an average time for complete clinical recovery from a moderately severe attack

The prognosis is good, complications are rare and unimportant, and the mortality in the acute stage is low—about 0 4 per cent Unfortunately in fatal cases there is little to show the ultimate outcome, as a rapid and unpredictable worsening of symptoms may be followed by death within twenty four hours Delirium and deep persistent jaundice are bad prognostic signs

Children

In children, amongst whom the disease is much more common, it runs a similar course except that it is, as a rule, very much milder The onset is usually more abrupt but with the same signs and symptoms as the adult Jaundice appears about five days later and is accompanied by a marked subjective improvement in the patient's condition It lasts for about ten days Death is very rare in the acute stage

In a very high proportion of children the icterus is fleeting, and the child feels perfectly well by the end of a week from the onset They are often allowed to return to school at this stage but it is very probable that they are still infectious, and so should be isolated for at least a fortnight from the beginning of the attack

Non-icteric Cases

Some cases, usually mild, run their course without ever developing jaundice but in the absence of a specific test for infection there is no evidence as to their frequency Liver function tests carried out during epidemics seem to indicate that they are numerous In all cases the urine of contacts should be watched for the appearance of choluria, as it frequently is not practicable to carry out the more complicated biochemical tests These non icteric cases are infectious and they represent a formidable epidemiological problem

Sequelæ

Relapse is relatively common Sometimes the attack is more severe on the second occasion, although icterus does not always recur It may come on at the end of convalescence and is particularly liable to occur if the patient has been permitted to return to work too soon The liver is tender on palpation and the biochemical liver function tests become abnormal again

In some cases a "post hepatitis syndrome" has been described with continuing anorexia, lassitude, mental depression and epigastric discomfort The evidence for it is largely subjective as liver function tests are usually within normal limits and biopsy reveals an essentially normal liver

Chronic hepatitis is an uncommon sequel, but a small percentage of patients show only partial recovery with continuing or renewed anorexia, an enlarged liver and abnormal function tests

The incidence of cirrhosis after infection is unknown but the available evidence suggests that it is not high

Congenital fœtal defects have been occasionally described following an attack of maternal hepatitis in the early months of pregnancy

TREATMENT

The treatment is symptomatic and the most important factors are adequate rest and diet Fluids with glucose added

are beneficial in the acute phase Once appetite returns a high protein and carbohydrate diet may be given and fats should be gradually increased during convalescence It must be remembered that most patients have lost weight and in some this loss may be marked Tests with methionine, choline and vitamin B_{12} have not as yet given any clear evidence of their being of value, but further trials are needed to evaluate them properly

As soon as the jaundice has disappeared and the brom sulphalein retention test is within normal limits, the patient may be allowed out of bed, gradually and for short periods at first, the subsequent course depending on the individual patient Alcohol is probably best avoided for about three months following an attack but there is no real evidence that it is harmful

In no circumstances should a patient be allowed to donate blood for at least twelve months after recovery

DIAGNOSIS

During the pre-icteric phase the diagnosis is often one of great difficulty, which is frequently only solved by the onset of choluria, or if this be missed, of jaundice itself No specific test for infection has yet been evolved—complement fixation tests have been described but are of little value

There is a characteristic leucopenia in the early febrile stage, and later a relative lymphocytosis Many of the lymphocytes are abnormal and similar in appearance to those found in infectious mononucleosis A lymphocytic response is, however, common to many virus infections and cannot be regarded as sufficient grounds for a diagnosis of any one of them The sedimentation rate is normal in the early stages and only rises with the onset of jaundice Bilirubinuria appears about the third day —before the serum bilirubin is raised Towards the end of the attack—about the end of the fourth week—it disappears, again oddly enough, before the

hepatitis also, the antibody is adsorbed by human liver or boiled guinea pig kidney

In Weil's disease a polymorphonuclear leucocytosis is present, a considerable albuminuria is frequent and the titre of leptospiral agglutinins rises sharply during the second week, at the end of which it may be as high as 1/1000 The condition is generally more severe, and the patient more ill than is the case with hepatitis It must be remembered that some 50 per cent of cases of Weil's disease do not have jaundice. The occupation of the patient, and the occurrence of spirochætal jaundice in his area should also be considered

Other causes of jaundice such as the hæmolytic anæmias may be outruled by clinical examination and the appropriate laboratory tests

PATHOLOGY

Our knowledge of the pathology of the condition owes much to the aspiration biopsy technique Dible, McMichael and Sherlock have examined a large series of specimens from cases of infective hepatitis, jaundice following arsenic therapy, and serum hepatitis Histologically all these conditions give fundamentally the same picture, which varies according to the nature and severity of the condition The picture is one of necrosis of the liver parenchyma, most marked at the centre of the lobule, and a cellular infiltration of the portal tracts In the milder cases or during recovery the infiltration with mononuclear cells is prominent, but all variations of the picture may be encountered The longer the jaundice lasts the more marked is the central necrosis In a few cases there is a residual fibrosis in the portal tract

An important factor in the recovery of the liver from what appears, histologically, to be severe damage out of proportion to the physical signs, is the preservation of the reticulin framework of the lobule In those cases in which it remains intact full recovery may be expected

In fatal cases the appearance varies according as death occurs in the acute stage or later In the acute phase the liver

is small and soft, and microscopically gives the picture of acute yellow atrophy The liver tissue is almost completely destroyed with no evidence of regeneration, and there is an intense peri-portal cellular reaction If death is delayed until the subacute stage, three to six weeks after onset, there is a firm granular liver, with destruction of the liver cells and infiltration and fibrosis of the portal tract There may be ample evidence of regeneration

At post-mortem a slight congestion or catarrhal inflammation of the stomach and duodenum, an enlarged spleen and abdominal lymph glands are found Ascites is an almost invariable finding

PROPERTIES OF THE VIRUS

Two strains of virus, recently isolated by Henle and his colleagues in tissue cultures and chick embryos, have proved capable of inducing mild hepatitis, without jaundice, in human volunteers Although it may be too early yet to identify these with certainty as strains of infective hepatitis virus, the evidence in their favour is very suggestive

No laboratory studies on the physical characteristics of these agents have yet been described, but from earlier experiments with infected materials certain properties could be ascribed to the infectious agent It is readily filtrable through Seitz or Chamberland filters, but no more critical studies to determine its particle size have been reported The virus is resistant to heat and cold, withstanding 56°C for thirty minutes and freezing for one and a half years More important, from a public health point of view, is its ability to withstand, for more than thirty minutes, one part per million of chlorine in water

EPIDEMIOLOGY

In man the virus is present in the blood during the acute phase, and it has been demonstrated there three days before,

and eight days after, the onset of an attack The length of its presence in the fæces is unknown, although this is obviously a point of considerable importance in any attempt to determine the duration of infectivity It is known to be present in the acute phase, but attempts to transmit infection to volunteers by filtrates of fæces which have been collected one month after the onset of an attack have been negative

Judged by its epidemic occurrence the geographic distribution of the disease is world wide Only in Denmark and Sweden, and since 1948 in Ireland, is the disease notifiable so that except for these countries little statistical evidence is available An interesting point about the Irish figures is that they show cases occurring in only three or four centres at any one time, the rest of the country being free of infection This state of affairs lasts for several months, but as soon as an epidemic has died down in one area the disease then appears elsewhere

Cases may occur at any time throughout the year The peak incidence is usually, but not invariably, in the late autumn and winter months One outbreak in Palestine reached a peak in June and another amongst school children in Ireland, in May

Age Distribution

Amongst civilian populations the disease is predominantly one of children, particularly those of school age Children under five years usually escape In England the attack rate during an epidemic in a population of 3 800 was 6 per 1,000 for the general population compared with 54 5 per 1,000 for school children In an Irish outbreak in a rural population of 2 500 the difference was even more striking—45 per 1,000 for the general population and 206 per 1,000 for the school children at risk Unfortunately for the statistician there is a high proportion of mild and fleeting cases in children which are never traced

More accurate figures for adults can usually be obtained due to the greater severity of the disease amongst them The

incidence appears to drop sharply after thirty years of age, probably due to immunological factors—such as an unnoticed or forgotten attack in childhood

Methods of Spread

Information as to the method of spread of the condition is derived almost completely from studies carried out during epidemics Here there is a marked divergence between the reports of military outbreaks on the one hand, and civilian on the other The bulk of the evidence in army cases points strongly to spread by the intestinal oral circuit, while amongst civilians spread by personal contact, probably by means of infected droplets, seems to be more important Generally speaking, in the latter close contact, such as occurs in schools and families, is necessary for infection, mere casual contact rarely being sufficient The infectivity of the condition is, therefore, low Explosive outbreaks have been described but the more usual curve shows a slow build up, the epidemic in any one area lasting three to four months No accurate data are available for the proportion of anicteric cases during an outbreak, but the proportion is high

Outbreaks have been reported which have been caused by the ingestion of infected milk, food or water, and in which it was possible to trace the infection to fæcal contamination of these vehicles

Although the inoculation of as little as 0 01 ml of infected blood has produced experimental infection, there is no evidence to incriminate insects The world wide distribution and the lack of seasonal specificity are also against this method of spread

Control

Any system of control must obviously be based on the two types of spread postulated for the disease Although no satisfactory experimental evidence has yet been brought forward to prove transmission by infected droplets, it would be foolish to ignore the numerous clinical observations on this point The presence of virus in the fæces rests on sound

experimental evidence. A case is generally regarded as being infectious for at least three days before and eight days after the onset of symptoms, and Pickles advocates the isolation of cases for fourteen days, a period which has proved safe in his hands. On occasion this period may not always be sufficient. The writer has seen one case which was infectious on the thirteenth day of her illness.

When, as is frequently the case, an outbreak is centred on, or occurs in, a school two methods of aborting an epidemic are available. In day schools in rural areas it would be wisest to close them as early as possible for at least a fortnight. Some further cases may occur but the epidemic will be checked. Little is to be gained by closing urban schools. In residential schools, once it is clear that an epidemic is on the way, the protective effect of gamma globulin during the incubation period should be tried. The dosage is 0.1 ml per pound body weight up to a maximum of 10 ml. Gamma globulin may also be of value in general practice and for the protection of contacts. It should be given as early as possible in the incubation period.

As the virus is known to be present in the fæces it is obvious that all possible means should be taken to avoid spread by this route, by ensuring that fæcal contamination of foodstuffs and eating utensils be prevented. In those areas such as the Mediterranean in which the disease is endemic, proper sanitation should be strictly enforced.

An interesting point which has been occasionally noted is the prevalence of hepatitis in scarlet fever wards in hospitals; this relationship has also been observed in general practice. From the epidemiological viewpoint it might repay further investigation.

IMMUNITY

It is probable—although not yet certain—that a high degree of immunity follows infection, a hypothesis in keeping with the decline in incidence in those over thirty years of age. The question is complicated by the clinical mildness of the

condition in the majority of children, and by the lack of specific serological test *for infective hepatitis* The protecti action of gamma globulin, when administered early in t incubation period, is in favour of such immunity Uncom monly, well authenticated cases of second attacks, as distin from relapse, have been reported

There is no information as to whether different strains infective hepatitis virus exist, or *of any cross immuni* between them if they do There is no cross immunity betwe infective hepatitis and serum hepatitis

SERUM HEPATITIS

Serum hepatitis is unique in its epidemiology It is, far as we know, the only infectious disease to be transmitte by *entirely artificial means*—*en passant* one may remark th it is almost the only disease which we insist on spreadin ourselves It is also unique in that while giving rise to n symptoms in its original host as far as can be traced, it wil nevertheless, produce a hepatitis in its new host The incuba tion period *is long—ranging from 40 to 160 days* with a average time of about 90 days

Clinically the picture is very similar to that of infectiv hepatitis but the onset is more insidious It tends also t be more severe, as it occurs more frequently in old an debilitated patients

The pathology of the condition is similar to that of infec tive hepatitis

Every case can be traced to an injection of human blood o its derivatives, usually received about three months pre viously. As little as 0 01 ml can initiate infection During the war more than 50,000 cases in the United States Army were traced to the inoculation of yellow fever vaccine which had been suspended in human serum Following on this the use of serum was dropped and no further cases have been

a relatively common occurrence in large clinics or other places where numbers of patients are treated, sometimes without proper sterilization of needles and syringes. Adequate sterilization by heat is the only effective safeguard, as the virus is peculiarly resistant to chemicals.

In the preparation of materials for injection—particularly serum or plasma or their products—care must be taken to prevent widespread dissemination of the virus. Pools of serum should, therefore, be kept as small as possible. Ultra violet irradiation of the serum or plasma has now been shown to be of little value as a method of sterilization. Filtration is of even less value as the virus readily passes through ordinary bacteriological filters.

Little is known of the properties of the virus and our information is derived from experiments on human volunteers.

There is no cross immunity between the viruses of infective hepatitis and serum hepatitis and the evidence for even homologous immunity following an attack of serum hepatitis is conflicting. It seems most likely that one attack will protect against subsequent infection.

The methods of transmission of the virus seem to be entirely artificial. It is spread by the injection of infected blood or blood products or alternatively by contaminated needles or syringes. The virus is not present in the faeces during the acute stage nor is there any satisfactory proof of its presence in the nasopharynx.

Cases amongst contacts have been reported but they are very rare. There have been no studies carried out to determine whether they were in fact secondary cases of serum hepatitis or coincidental cases of infective hepatitis. Even if parenteral inoculation is not the only method of spread it is certainly the most important.

The virus is present in the blood during the acute phase and also in the incubation period and in one case has been recorded eighty seven days before the onset of jaundice. It has not been demonstrated during convalescence and in some experiments could not be recovered one month after onset.

We do not know whether a chronic stage exists nor what, if any, proportion of cases become carriers.

No case should be allowed to donate blood for two years, if ever, after an attack.

Chapter Fourteen

YELLOW FEVER

The story of yellow fever is one of the most fascinating in medicine. The interest of its early dissemination along the slave trade routes from its home in Africa is paralleled by the work of Reed and his colleagues in the early days of this century, when the importance of mosquitoes in the spread of the disease was first recognized. After this discovery it seemed that by the destruction of *Aedes* mosquitoes yellow fever could be readily eliminated from the New World. Then the recognition, first in America and later in Africa, of jungle yellow fever as an entity distinct from the classical disease made it necessary for the epidemiologist to start again almost from the beginning. As against these setbacks may be placed the tremendous advance made by the introduction of a safe and efficient form of prophylaxis. This has made it possible not only to envisage control of the disease in endemic areas but also to protect those travelling to them. It has also made it possible for the laboratory worker to handle the virus with complete safety.

CLINICAL FEATURES

The incubation period is generally four days from the bite of an infected mosquito with three to six days as outside limits.

The onset is acute with rigors, back and limb pains and a rapidly rising temperature which may reach 104° F. The pyrexia is at its height by the first or second day, and is initially accompanied by a rapid pulse. This falls steadily as the temperature rises—a finding typical of yellow fever which is known as Faget's sign. In this first stage of

congestion or initial fever, during which virus is present in the blood, the patient is obviously ill and restless, with flushed face and injected conjunctivæ Nausea, vomiting and constipation are present and continue throughout the illness After two or three days the temperature falls A day or so later it rises again, and the appearance of the patient changes to one of a dusky pallor Jaundice now appears and there is a definite *hæmorrhagic tendency with some vomiting of altered* blood (' black vomit), melæna and ecchymoses The pulse rate falls steadily and may reach 50 per minute or lower The restlessness of the first stage gives way to lassitude and depression

Albuminuria, which is present from the second day, is marked at this stage and numerous casts are found microscopically

In the early stages there is a pronounced polymorpho nuclear leucocytosis which falls steadily, until by the fifth or sixth day there is a definite leucopenia affecting mainly the polymorphs

Blood is present in the vomit and the stools

In severe cases a third stage supervenes with vomiting of altered blood, anuria, and delirium and convulsions prior to death A falling temperature and rising pulse rate are bad prognostic signs Death is commonest on the sixth or seventh day and is rare later than the tenth day from the onset In *the African native the condition is rather milder* and death, when it occurs, is later

Many cases make an uneventful recovery from the second stage The temperature returns to normal about the eighth day and the albuminuria gradually diminishes Complications are *very rare One attack confers a life long immunity*

These signs and symptoms are those of classical yellow fever and many cases, probably the majority, will show few of them, presenting merely with headache and slight fever

The mortality rate reported varies enormously—from 5 per cent *suggested as an overall figure to 60 or 65* per cent men tioned in some outbreaks As mild infections become increasingly recognized the figure will certainly fall

TREATMENT

There is no specific treatment

PATHOLOGY

On inspection of the body definite jaundice is present and there may be ecchymoses under the skin Hæmorrhages are found in the heart, lungs, brain and intestines and particularly into the mucous membrane of the stomach

The most obvious changes are found, as might be expected in the liver which shows severe damage To the naked eye it is slightly enlarged and fatty On microscopical section widespread necrosis and fatty degeneration are found The centre of the lobule is the area of most striking damage, but there is a layer of intact cells surrounding the central vein and a thin rim of normal cells may be found at the periphery Scattered throughout the affected areas are cells showing hyaline necrosis—the so called Councilman bodies Eosinophilic intranuclear inclusions, which are frequent in the experimental disease in monkeys, are rare in man

In spite of these widespread changes the normal structure of the liver is still preserved, and in those who survive it recovers completely with no sequelæ It is obvious that the microscopical findings are very similar to those of infective hepatitis and considerable experience is needed to differentiate between them

The kidneys show fatty degeneration and necrosis of the tubular epithelium together with numerous casts—hyaline, granular and calcareous The latter are said to be diagnostic

Irregular patches of fatty degeneration are also present in the heart but these changes are not pathognomonic of yellow fever

LABORATORY DIAGNOSIS

Very frequently it is impossible to make a confident diagnosis without laboratory aid Of the methods available

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LABORATORY DIAGNOSIS

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one, histological examination of the liver in fatal cases, has been already considered More *satisfactory are the isolation* of the virus from the blood, or the demonstration of a rise in antibody titre

In attempting isolation blood should be taken, daily if possible, during the first three days of illness and certainly not later than the fifth day This is inoculated into rhesus monkeys or white mice

The demonstration of an antibody depends on the marked difference in the titre of neutralizing antibodies in two specimens of blood—the first taken early in the disease and the second two or three weeks later The sera are titrated by the intraperitoneal or intracerebral inoculation of virus serum mixtures into mice

EPIDEMIOLOGY

It is now well established that from the seventeenth century onwards sailing ships, by the carriage of infected mosquitoes, played an essential part in the spread of yellow fever from Africa In retrospect many epidemics in temperate climates can most probably be attributed to them Outbreaks occurred in Swansea and Southampton during the 1870's In America even northern ports were subject to frequent outbreaks which reached a peak during the summer months and died away during the winter The last large outbreak in the United States occurred in New Orleans as late as 1904

Nevertheless, for several hundred years the method by which yellow fever was transmitted remained a complete mystery, and many fierce controversies took place amongst the earlier epidemiologists It was not until 1881, when Finlay suggested that infection was spread by mosquitoes that the scientific study of its epidemiology may be said to have begun The definitive proof of the idea of an inter mediate host was provided by Reed and Carroll in 1900 The essentials for the continuance of yellow fever in any one area were then realized—there must be a constant *supply of*

susceptibles and a sufficiency of the insect vector By attacking the latter the disease was rapidly eliminated over a great part of North and South America in the first twenty years of this century

Carriage by Mosquitoes

The insect vector implicated was that mosquito which is now known, after several changes of name, as *Aedes aegypti* It is a domestic insect insofar as it is found in the vicinity of dwelling houses The female lays her eggs in clean water such as occurs in barrels, drains, cans, and other vessels in which water may fortuitously lodge, and which are common near houses Such habits also explain why the mosquito, and therefore the disease, travelled with sailing ships—in water containers—through the centuries Other mosquitoes have since been found to act as carriers, and are of importance in the spread of the recently discovered jungle yellow fever, but the classical urban, or epidemic, disease is associated with *A aegypti* alone

The behaviour of yellow fever virus in the mosquito has been extensively studied, without any very definite conclusions being reached It does not multiply to any great extent in its insect vector, nor does the mosquito act as host for any essential part of its life cycle The insect is of importance as a medium of transmission rather than of growth, but as such is ultimately essential for the survival of the virus Experimentally it has been shown that a mosquito can transmit the virus after a variable period of days or weeks following infection This time depends on the temperature of the environment The higher the temperature the sooner a mosquito becomes infective

Immediately after infection the titre of virus in the insect is very high, but it subsequently falls to rise again and reach an infective level later It is suggested that the fall and subsequent rise are due to destruction of virus by the tissues, followed by a period during which the virus multiplies and ultimately again reaches an infective level The de

multiplication in any given mosquito seems to be largely a matter of chance, which would explain the conflicting reports on this particular point It seems reasonably certain that, while an infected mosquito remains infectious for life under natural conditions, there is no transmission of infection from one mosquito to another, and that infection is not transmitted to the larvæ

Types of Yellow Fever

Extensive investigations have shown that yellow fever as it occurs in Africa is identical with the condition in America, and similarly that the disease is the same no matter how transmitted The difference between methods of spread, which will be discussed are of great importance from the point of view of control, but are of no significance clinically or immunologically There is only one disease caused by the yellow fever virus

Nevertheless, several epidemiological types of the disease are distinguished, depending on their methods of transmission and their animal reservoirs These may be classified as follows —

(1) The classical *urban* yellow fever occurs in epidemic form It is that described historically, and is still of great importance today Infection is transmitted by *Aedes aegypti* mosquitoes and the cycle is *man—aegypti—man*

(2) Rural or *jungle* yellow fever is the hard core of the problem now facing the epidemiologist There is a reservoir of the disease in jungle animals, not yet identified with certainty but probably monkeys *A aegypti* is not essential for the continuation of the infective cycle, other varieties, principally of the *Hæmogogus* species, being more usually responsible Man is merely incidentally involved, only those who enter the jungle or live on its outskirts being infected Its importance, apart from rendering wide areas virtually uninhabitable, is that any time a classical urban epidemic may arise from the jungle cycle, as happened in South America in 1937

(3) Rural outbreaks due to *A aegypti* have been occasionally reported from sparsely populated areas in which water is scarce, and in which each household has its own water storage which supplies breeding grounds for the mosquito This type is of little significance

For control purposes on an international basis the World Health Organization has divided yellow fever areas into the following categories —

(a) *Enzootic* yellow fever areas, free of *Aedes aegypti*, in which the virus is present and persists amongst animals for long periods of time, with the production of accidental human infection (woodcutters, hunters, etc), only

(b) *Epizootic* yellow fever areas, free of *Aedes aegypti*, in which the disease occurs periodically amongst animals for short periods of time

(c) *Endemic* yellow fever areas, with *Aedes aegypti*, in which the virus is present and persists amongst animals for long periods of time

(d) *Epidemic* yellow fever areas, in which cases caused by transmission of the virus by *Aedes aegypti* occur

As will be seen from these definitions the fundamental difference between an endemic and an epidemic area is that transmission from man to man by *A aegypti* is potential in one, and is observed in the other

America

The area in which yellow fever occurs in America was steadily reduced over a period of years by the rigid supervision of possible breeding grounds of *A aegypti*, but it still comprises a large area in the tropical zone of South America It seemed certain at one time that the disease existed only in a few endemic foci in the coastal regions, and that its eradication was a matter of time Then cases deep in the interior, far from any known source of yellow fever, were recognized Finally the description of an outbreak in which *Aedes aegypti* could be eliminated as a vector, drew attention to the entity of jungle yellow fever

The World Health Organization agrees to certain ports and other areas being considered as no longer endemic regions provided the *A aegypti* index does not exceed one per cent This index, which is used as a basis of control, is the percentage of dwellings in any given area in which larvæ of the mosquito are found breeding It has been found more practicable to eradicate them completely than to maintain a critical level below which transmission would not occur In fact, the mos quito has now been wiped out in most of the ports and cities of South America Before the introduction of efficient methods of vaccination such anti mosquito measures were the only practicable ones They can now be supplemented by the immunization of those persons liable to contact infection

Constant supervision is, of course, needed The last classical *A aegypti* transmitted epidemic arising from a similar cycle was in 1934, but since then, in 1937, a classical epidemic secondary to jungle yellow fever occurred Such a superimposition upon an outbreak of the jungle type is always liable to occur if the measures designed to eradicate the mosquito population are not rigorously and continually enforced Similarly, jungle yellow fever is being recognized in "new" areas *An outbreak occurred in Panama in 1948* No clinical case of the urban variety had been recognized there for fifty years

A further weapon of control—the viscerotome—is also widely used in South America As its name implies, this is an instrument designed to obtain a specimen of liver without the necessity of performing an autopsy Since its introduction many thousands of examinations have been carried out, and legal sanction now permits the taking of such specimens from any person dying of pyrexial illness of less than ten days' duration The information derived has been of the greatest epidemiological value in demonstrating the existence of yellow fever in areas in which it was unsuspected and, con versely, in demonstrating freedom from infection for long periods of time in other areas

Africa

The epidemiology of the disease in Africa presents even more formidable problems than it does in America. The yellow fever belt extends right across tropical Africa between 15° north and 10° southern latitude with the exception of Tanganyika. An area of Northern Rhodesia is also infected.

In the home of classical epidemic yellow fever the distribution of *A. aegypti* is far more widespread than in America and less information is available about it. In addition there is gradually accumulating evidence that jungle fever occurs and that other varieties of mosquitoes are involved in its spread. One of the largest outbreaks of recent years—in the Nuba Mountains of the Anglo Egyptian Sudan in 1940—was of the jungle variety and although it was present *A. aegypti* did not play a very prominent part in its transmission.

PROPERTIES OF THE VIRUS

Filtrability

The virus is readily filtrable as was shown by Reed and Carroll as far back as 1902 when they passed it through a Chamberland F filter. It was subsequently shown to pass through Berkefeld V and N and Seitz FK filters. For many years complete failure to obtain positive filtrates from suspensions of mosquitoes was reported and it was thought that the amount present was too small to allow for the inevitable loss by adsorption on to the filter. It is now known that the real reason was that the virus is inactivated rapidly by saline and positive results have since been obtained by substituting serum for it.

Size

By filtration experiments the virus has been shown to have a diameter ranging from 17 to 28 $m\mu$.

HEAT AND COLD

The virus is inactivated by exposure to 65°C for ten minutes It will survive for five weeks in blood stored in a refrigerator and, if frozen and dried *in vacuo*, remains viable in infected tissues for two years

CHEMICALS

Various chemicals such as dilute formaldehyde, ethyl alcohol, and phenol rapidly inactivate the virus It is resistant to glycerol, and will survive for 100 days in a 50 per cent solution

Strains of Yellow Fever Virus

As it occurs in nature yellow fever virus is pantropic, that is it attacks all three embryonal layers On continued transmission in various laboratory animals, strains can be divided into *viscerotropic*, which affect principally the liver, and *neurotropic* It must be realized that such divisions are not absolute, as both varieties can be further modified experimentally Viscerotropism is well demonstrated in the monkey, and neurotropism in the mouse

Experimental Animals

The discovery by Stokes, Bauer and Hudson that the *M rhesus* monkey is susceptible to infection with yellow fever virus was a turning point in the history of the disease When this discovery was made it then appeared that all the essentials for the control of yellow fever were known The disease was later found to be transmissible to many other species particularly to Indian monkeys African monkeys seemed to be generally resistant and this was thought to be due to a natural racial immunity In the light of the subsequent discovery of jungle yellow fever it is clear that some monkeys may be immune, not because of any racial immunity, but because of previous infection Monkeys may be infected by parenteral inoculation such as by the injection of minute quantities of blood, or the bites of infected mosquitoes

Other animals which may be used are mice and guinea pigs Mice are infected by intracerebral inoculation This gives rise to an illness which caused by yellow fever virus shows neither the clinical nor the pathological features of human yellow fever and modifies the virus so as to make it less virulent when transferred to monkeys

Some animals such as rabbits and rats, are quite resistant to infection

The virus grows well in tissue cultures Pantropic strains are difficult to establish but neurotropic strains propagate readily Once again there is a modifying effect on the virus The 17D strain which is used for vaccination was originally produced after several hundred passages in tissue culture using various non nervous tissues of the chick embryo On subcutaneous inoculation this strain gives rise to a mild systemic infection with the production of antibodies Inoculated intracerebrally into monkeys less than 5 per cent will develop encephalitis Chick embryos are also widely used for the growth of the virus in the laboratory and at present are the principal source of yellow fever vaccine

IMMUNITY

Infection if not fatal gives rise to neutralizing antibodies which are protective for life Complement fixing antibodies also appear but seem to be of little significance

Antibodies may be demonstrated by means of the mouse protection test Essentially this consists of the intraperitoneal inoculation of a mixture of virus and the serum to be tested into mice By means of it widespread surveys of populations have been carried out, and it is one of the most valuable weapons at the disposal of the epidemiologist

CONTROL

The two pillars on which the control of yellow fever rests re anti mosquito measures and vaccination

Anti-Mosquito Measures

These have been concentrated in the past on the destruction of the larvæ and the elimination of possible breeding grounds D D T and similar preparations have enormously increased the possibilities of control as a single spraying renders walls *and other structures lethal* to mosquitoes for several months

Vaccination

The introduction of safe and efficient methods of vaccination has completely changed the problem *presented by yellow* fever to the epidemiologist and the research worker Previously the risk to laboratory workers was appalling as the number of deaths amongst them demonstrates In addition travel into and out of endemic or epidemic areas is now possible without the risk of contracting or of spreading infection Two methods are at present extensively employed

17D Strain

The origin of this strain has already been mentioned After subcutaneous inoculation antibodies appear about the sixth day and protection lasts for six years and possibly for longer The international certificate against yellow fever at present recognizes a period of only four years but this will probably be extended in the near future The vaccine consisting as it does of live virus gives rise to a mild and modified attack *of yellow fever but apart from those sensitive* to egg proteins as the vaccine is now prepared in chick embryos less than 5 per cent of those inoculated develop a mild reaction about the seventh day In the majority no reaction whatever occurs

During the war the efficacy of the vaccine was put to a very severe test with eminently satisfactory protective results Thousands of cases of jaundice *following inoculation* with the vaccine were reported but these were traced not to the *strain* of yellow fever virus used but to icterogenic human serum in which some batches had been suspended Since the

use of human serum as a vehicle was abandoned, following on this occurrence, no cases of jaundice attributable to the vaccine have occurred

[D]akar Strain

This strain, derived from infected mouse brain, is extensively used by French workers Suspended in gum acacia it is inoculated by scarification, and it is often combined with vaccinia virus, so that the two vaccinations are carried out simultaneously The titre of circulating antibodies evoked by it is higher than by the subcutaneous inoculation of the 17D strain, but on the other hand some 15 per cent of those inoculated develop reactions—few of them, however, severe

It can be fairly claimed that tremendous strides have been made in the control of yellow fever by placing it on an inter national basis The carriage of the disease by persons travelling from infected areas is now easily controlled by vaccination and quarantine

The possibility of transporting infected mosquitoes is always present, and whilst the lovers of the romantic may deplore the eclipse of the sailing ship, the epidemiologist may equally rejoice The modern steamship, with no water casks and with proper ventilation, is much less likely to act as a carrier of infection Aircraft obviously need attention That the measures in force for the protection of aerodromes and aircraft themselves are well founded, is shown by the fact that there is no case on record of the transport of yellow fever by air

It must always be remembered that there are wide regions, notably India, with millions of susceptibles and large populations of mosquitoes capable, if once infected, of spreading the disease Obviously, therefore, only the most rigid precautions can prevent a possible catastrophe

Chapter Fifteen

RABIES
(Hydrophobia)

Rabies has been stamped out in Great Britain, Irel
Scandinavia, and several other western European count
but it is still a formidable problem over most of the wo
Primarily a disease of wild carnivora it spread to dome
animals some two centuries ago, and has never since b
completely eradicated

CLINICAL FEATURES

The incubation period is variable and is dependent
many factors It was previously thought that the m
important factor was the location of the bite, it is now kno
to be the amount of virus introduced into the body at t
time of the bite The delay before the onset of symptoms
shorter in cases of biting around the head and face than it
for the limbs and so it was considered that the distance t
virus had to travel to reach the central nervous system w
the governing factor Recent experimental work shows th
this theory was incorrect Biting wounds tend to be mo
severe around the face with its abundant innervation ar
superficial musculature, than on the limbs which, in additio
are frequently protected to some extent by clothing

Following the bite of a rabid animal, symptoms appe
any time between fifteen days and five months later in th
great majority of cases, although instances, shorter or longe
have been reported outside these limits

Hydrophobia

A well defined prodromal period ushers in the attack It is characterized by pyrexia pain at the site of the wound, even though it may be apparently completely healed, headache, restlessness, and pains in the back and limbs At *first hydrophobic attacks occur only on attempting to take* liquids—the head is thrown back and the liquid is forcibly ejected on coming into contact with the fauces Later, such spasms are induced by almost any stimulus such as loud noises or draughts There is marked dyspnœa and Cheyne Stokes breathing may occur Saliva dribbles out of the mouth as the patient tries to avoid swallowing it During the attacks the patient is restless, and even maniacal, and tries to bite those near him, between them he is anxious and has often a feeling of impending death Paralysis, or paresis, of groups of muscles may be found if looked for, but they are rarely prominent in such cases

Once hydrophobia occurs the disease is uniformly fatal

In occasional cases the clinical picture is paralytic rather than furious, or paralysis may supervene at any stage and subsequently predominate When the disease starts in this manner the clinical diagnosis is one of great difficulty

Once the symptoms develop there is no treatment of value except to attempt, by anæsthesia or other means, to diminish the frequency and severity of the spasms Barbiturates are reported to be more successful than morphia in the anxious phase

PATHOLOGY

On inspection the cord and brain are congested, and the latter shows a moderate degree of œdema

Histological changes are found in almost every area of the central nervous system Congestion and neuronal damage is seen in the cerebral and cerebellar cortex and is severe in the mid brain, basal ganglia and pons The most marked changes are found in the medulla Some degree of interstitial

cell infiltration occurs throughout the central nervous system Its severity depends on the length of the disease, and it is most intense in long standing cases Thrombi may be found in the vessels of the affected area

Similar changes are found in the cord and if a limb has been bitten, they are very marked in the corresponding segment, the posterior horns being most affected Axons and myelin sheaths are degenerated, the former appearing beaded and the latter vacuolated

Changes may be found in individual neurons all through the nervous system The Nissl substance is lost and the cytoplasm degenerated, the process ending in neuronophagia

The lungs are atelectatic and the mucosa of the bronchi and trachea show congestion

Changes may occur in the salivary glands depending on whether or not virus is present in the saliva If it is, the acinar cells show marked degeneration and there is a lympho cytic infiltration of the interstitial tissue

Negri Bodies

In spite of the very marked histological changes throughout the central nervous system the only diagnostic finding is the occurrence of Negri bodies These are eosinophilic, intracytoplasmic bodies, which may be large or small depending on their position in the neuron In the human they vary in size from 2 μ to 10 μ They stain well with Mann's, Laidlaw's or Seller's stains The larger bodies have a baso philic internal structure, or granule, which morphologically closely resembles a nucleus The significance of these nuclear structures remains undetermined

Negri bodies frequently are not found at all in human cases When present, they are seen in greatest numbers in the hippocampus They may also occur in the pyramidal cells of the cerebral cortex, the Purkinje cells of the cere bellum the large neurons of the basal ganglia and cranial nuclei and in the spinal cord

The precise nature of the Negri body is unknown When first described they were thought, not unnaturally, to be protozoal in nature No agreement has yet been reached, and present day theories oscillate between their being colonies of the virus and a cellular reaction to it They may be, of course, and probably are, a combination of both—that is virus particles embedded in a matrix of degenerated cell elements In any case, whatever its nature, the Negri body when it is found is pathognomonic of rabies

PATHOGENESIS

Rabies virus is usually present in the saliva of an infected animal and is implanted in the body by biting The important factor in infection is not the actual bite itself, but the deposition of infected saliva which occurs with it Cases of rabies are on record where there was no history of a bite, infection was contracted through recent wounds which had been contaminated by infected saliva

After implantation by these means the virus apparently enters the damaged nerve endings in the region of the wound, and thence spreads by nerve trunks throughout the central nervous system No proper study of the role of the blood and lymph streams has yet been undertaken, but as far as is known at present they play little or no part in the dissemination of the virus in the body At any rate, it is clear from experimental work that the nervous system itself can act as a very efficient vehicle for its spread Experimental work has also shown that the primary tissue reaction to infection is a neuronal degeneration

After death the virus may be recovered from several sites in the body It has been found in the scar of the wound, but its presence there does not appear to be constant In the brain the thalamus and medulla frequently contain high concentrations and it may also be isolated from the spinal cord and the nerves It is not found in the cerebrospinal fluid

LABORATORY DIAGNOSIS

If a biting dog is suspected of having rabies it should, if it is not obviously hydrophobic, be caught and kept under observation It is a mistake to destroy it immediately this may leave no means of knowing whether or not the person bitten has, in fact, been exposed to infection In the early stages of rabies in the dog Negri bodies are scanty, and to make a definite diagnosis time consuming animal inoculations must be done If the dog is confined, instead of being destroyed, *much time which may be vital will be saved* An infected animal will die in two to three days of obvious infection, numerous Negri bodies on which to make a diagnosis will then be present, and treatment can be given to the patient within the first four days Should the animal survive for more than a week, it can be taken that there is no danger of rabies developing in the patient

If there are no diagnostic facilities in the neighbourhood, the dog's head should be packed in ice after death and sent to the nearest laboratory by the quickest possible route

The laboratory diagnosis in humans is, of course, always retrospective The hippocampus should be dissected out and smears or, better, impression preparations made from it These may then be stained by any of several methods, e g Mann's, Laidlaw's, Giemsa, or Seller's stains and examined microscopically for the diagnostic Negri bodies

Histological preparations should also be made from other parts of the central nervous system

It is also advisable to attempt the isolation of the virus by animal inoculation Suspensions in saline of the bulb and other parts of the nervous system are inoculated intracerebrally into rabbits, mice and guinea pigs, in which symptoms will develop after a varying incubation period

PROPERTIES OF THE VIRUS

Thus far nothing has been said of the measures available for prophylaxis in those who have been bitten by rabid

animals, because to understand their value it is necessary to know something of the properties of the virus The historical background of preventive inoculation is well known, being as it is linked with the name of Pasteur who carried out the first successful treatment

As it occurs in nature rabies virus is known as *street virus* By continued intracerebral passage in animals street virus is considerably modified in its properties, and it is then known as *fixed virus* (*virus fixé*) The two types of virus are differentiated by their effects on susceptible laboratory animals Some strains of street virus have a very high virulence and a short incubation period for laboratory animals, and are termed *renforcé* strains (*virus de rue renforcé*) Similarly strains of low virulence have been described, an example being *Oulou fato* which occurs in North Africa

Fixation of rabies virus is carried out by the intracerebral inoculation of rabbits Strains vary in the ease with which this occurs and an average of fifty passages is required Once fixed rabies virus is very stable in its characteristics By the end of the fixation period the effects of the street and fixed strains of virus on experimental animals are quite different but there remains a close antigenic relationship between them They share antigens in common The differences are due to quantitative variations amongst these antigens

The main differences between street and fixed virus may be summarized as follows on intracerebral inoculation into rabbits the incubation period changes from fifteen to twenty

or not at all with fixed virus Dogs infected with street virus develop hydrophobic or furious rabies whereas the dumb or paralytic disease develops with fixed virus After intracerebral or intraneural inoculation street virus is regularly disseminated throughout the central nervous system fixed virus is not so regular in its spread Most important of all

street virus is pathogenic for man, whilst fixed virus is al-
non virulent

TREATMENT

Immediately after the bite treatment of the wound sh
of course, be carried out There is very little evidenc
show that severe cauterization is of greater value in
venting the onset of rabies than is irrigation with :
antiseptics

The vaccine treatment of rabies goes back to Paste
discovery that virus in infected rabbit cords becomes
gressively less virulent on dessication The first case
treated by him as far back as 1885 The rationale of
treatment depends on the long incubation period, w
allows the immunization of a person bitten by a rabid ani
before clinical symptoms develop

Not every person bitten by an animal may require vac
treatment There is a slight, but definite, danger of compl
tions due to the vaccine itself, and each case must be
sidered individually In fact, the virus is actually presen
the saliva of only some 50 per cent of animals with clin
rabies, but, if present, may have been there for four d
before symptoms develop The question of treatment can
be made dependent on its presence or absence at the tim
the bite—the virus must be assumed to have been presen
all cases Statistics show that only between 5 and 15
cent of those bitten but untreated develop rabies but suc
finding is no justification for withholding vaccine treatm
in any individual case

The following general rules may be laid down —

(1) When the biting animal is found to have rab
treatment should be started immediately *The best resu*
are obtained if it is commenced during the first four days

(2) If, in an endemic rabies area, the dog concern
cannot be traced, or is destroyed *immediately so th*
laboratory investigations cannot be carried out, treatme
should be initiated as soon as possible

Many such cases will be treated unnecessarily but there is no excuse for denying the treatment to them

(3) If the animal concerned is identified and shows no clinical evidence of rabies infection it *should* be isolated for observation as it may be in the early stages of the disease. If so it will become clinically obvious within two or three days—in time to allow of successful vaccine treatment—and the diagnosis can be confirmed by post mortem examination of the animal s brain If the dog does not develop rabies within seven days it can be taken that it was not infected

(4) Cases arise from time to time in which a person has been in contact with a rabid animal but was not bitten These cases are difficult to evaluate If there are recent injuries or the continuity of the skin is broken and infected saliva may have been deposited in this area treatment should be given The mere fact of being in contact with rabies is not of itself an indication for treatment

The original vaccine used by Pasteur which was prepared by the dessication of rabbit cords contained living fixed virus which is of reduced virulence for man This vaccine is still in use in France and the French colonies, as well as in several other countries In most countries nowadays how ever a phenolized vaccine first introduced by Semple is preferred The reason for this is that a number of so called laboratory accidents followed the administration of rabies vaccines and as remarked earlier their use is not entirely without risk

The most serious complication is the so called *rage de laboratoire* which is uniformly fatal and is due to the living fixed virus present in the dessicated vaccine Its incidence is very low but it does not occur at all with the killed virus in the phenolized vaccine

With either type of vaccine neuro paralytic accidents may supervene during treatment The pathology of this con dition which is characterized by varying degrees of paralysis differs from that of rabies. No Negri bodies are found in fatal cases and no virus has ever been isolated from them Its

relationship to the other post infective encephalitides is obscure, as the characteristic perivascular demyelination is rarely found It has been *suggested that, in the case of* phenolized vaccines, the normal nervous tissue present in the vaccine itself is in some way responsible In those vaccines containing live virus the most likely ætiological agent appears to be *virus fixe*

"Rage de Laboratoire"

This comes on soon after a course of treatment is finished It begins with paralysis of the lower limbs which spreads rapidly No hydrophobia is seen, nor are Negri bodies found after death, but *virus fixe* can be readily isolated from the brain

"Neuro-paralytic" Accidents

These may occur on the thirteenth to the fifteenth day of treatment *Varying degrees of paralysis are seen, from* a spreading picture to paralysis of localized groups of muscles The former carries a mortality rate of some 30 per cent but the prognosis is good in the other varieties

Treatment by dessicated cord vaccines is carried out by daily injections for fifteen to twenty five days depending on the severity of the case The phenolized vaccines are injected daily for seven to fourteen days As distinct from the serious *complications, general reactions* such as pyrexia and headache occasionally occur There may also be an erythematous or an urticarial reaction at the site of the injection

One of the original objections put forward *to the use of* the phenolized vaccine was that the antibody response to it was not as good as with the dessicated cord vaccine Subsequent work has shown that it is equally efficient in that respect That being so, on general principles it would seem preferable to use killed virus in vaccines—even though the incidence of *rage de laboratoire* is so low as to make it almost a curiosity

Experimentally, vaccines in which the virus has been inactivated by ultraviolet light seem to be best of all, but very little information is yet available as to their effects in man

We are still uncertain as to how rabies vaccines act Rabicidal antibodies develop in the blood but the virus is in the central nervous system and their effect is doubtful It could be that the immunity produced by treatment is a tissue immunity in the central nervous system This may be produced by the "interference phenomenon," but there are no experimental findings as to the mode of action of the vaccine

Results of Treatment

These are very difficult to assess statistically, due to the number of cases which are lost sight of after a course of treatment is finished A proportion of these may subsequently develop rabies without its being known and such a case would be entered in the records as having been successfully treated In as many as a third of the treated cases the incubation period may be more than three months

A further complication is introduced by the *relatively* low incidence of rabies in untreated cases, which has been calculated at only some 5 to 15 per cent of those bitten by rabid animals Other factors must also be taken into account such as the severity or the location of the bite The real difficulty arises from the fact that it is impossible to say that any given case has been actually infected The infective saliva may have been absorbed by clothing and never have reached the wound Such a case, subsequently treated, would be regarded as a success for treatment even though never, in point of fact infected

Rigid statistical proof of the value of vaccines is therefore lacking On the other hand must be put the experience of more than sixty years, and from this it appears that vaccine treatment is almost certainly of value How *much protection* it gives or how the protection is evoked, is unknown, but such findings have parallels in other branches of medicine Of approximately 1 600 000 cases treated the incidence of rabies

in Europeans receiving treatment was 0 15 per cent, and in non Europeans 0 52 per cent In brief, therefore, although the value of treatment has not been statistically proved it must be given if the indications point to it

Immune Serum

A recent advance of great interest is the introduction of hyper immune serum in place of vaccination for the treatment of rabies in animals This change from active to passive immunization has given very good results It has only rarely been used in human cases, and in order to obtain statistical proof of its value, field trials, under the auspices of W H O, are to be carried out in Egypt and Israel

Chick Embryo Vaccines

Simultaneously with these trials, experiments will be undertaken to determine the efficiency of vaccines prepared from infected chick embryos It has been claimed that these give a higher and more lasting degree of immunity in dogs than do the vaccines previously used

EPIDEMIOLOGY

Rabies is widespread throughout the world A few countries have succeeded in eradicating it By enforcing rigid quarantine procedures over all animals entering the country, they have prevented its re appearance

In western Europe the fox is important as a reservoir of the virus In eastern Europe the wolf, and in Asia the mongoose, act as reservoirs Canada is free of infection but it is prevalent amongst foxes in areas of the United States, and in dogs in South America In general, the dog seems to be the most important single factor in keeping any area infected

All rabid animals, and such others as may be bitten by them should be immediately destroyed, with the proviso that those biting humans should, as already *mentioned, be* confined for observation for as long as may be necessary

In areas where rabies is endemic every dog bite should be reported to the public health authorities

Quarantine is of undoubted value and experience has shown that the six months period imposed in Great Britain and in Ireland is not excessive It must be rigidly enforced, as the disease was reintroduced to England, apparently by smuggled animals, after the country had been free of it for many years

Vaccination of dogs has been extensively carried out in some countries with satisfactory results To be effective it must be repeated yearly as immunity wanes at the end of that period Chick embryo vaccines seem to protect animals for longer than this, and they may replace those treated with chloroform or ultraviolet light which until now have been the vaccines of choice

PHYSICAL CHARACTERISTICS OF THE VIRUS

The Negri body has already been described

The virus is filtrable through the ordinary bacteriological filters such as the Berkefeld, Mandler or Chamberland Filtration experiments with collodion membranes have shown its size to be between 100 mμ and 150 mμ

It has been grown successfully in tissue culture, and in the various cavities of the chick embryo

Heating to 50°C for fifteen minutes destroys the virus and it is also sensitive to ultraviolet light, phenol and formalin It will survive in 50 per cent glycerol in a refrigerator for one year Dessication *in vacuo* preserves its virulence which is, of course, lost on dessication by other means

TRINIDAD RABIES

This variety of the disease is found only on the island of Trinidad It is spread by the vampire bat (*Desmodus rotundus murinus*) which normally feeds on cattle As a rule humans are only attacked if, for some reason, cattle are not available

Chapter Sixteen

POLIOMYELITIS

Poliomyelitis has a long history extending back as far as the fifteenth century B C but there is no record that the epidemics with which we are familiar today occurred before the middle of the nineteenth century During the past hundred years the pattern of the disease in many countries has changed from an endemic disease with occasional small outbreaks to an epidemic condition involving hundreds of cases every few years The change is striking but the reasons for it are not yet fully understood It is very probable as will be seen that man himself has by improved methods of sanitation profoundly altered the epidemiological picture of the disease

It has been suggested that the virus was originally an intestinal parasite of rodents which later became adapted to man Possibly some chance mutant was pathogenic for man and by contamination of foodstuffs or eating utensils gained an opportunity for attack thence a variant arose which could pass directly from man to man This hypothesis cannot be proved one way or the other but in its favour is the existence of a poliomyelitis group of viruses centred largely on rodents

It was at first thought that poliomyelitis virus was naturally pathogenic for man only and for monkeys and chimpanzees experimentally Recent work has differentiated a group of closely related viruses some of which occur naturally in mice and other rodents and others which are capable of being transmitted to these animals in the laboratory Our knowledge of the significance of these findings is not by any means complete The bulk of strains isolated from the human are transmissible to monkeys or chimpanzees only and are

referred to as "simian" A few, however, can infect rodents, of these the best known are the Lansing and the MEFI strains Others have been recorded, but their classification as typical human strains has not been so well authenticated

Amongst animals two diseases have been described which bear a marked similarity, clinically and epidemiologically, to human poliomyelitis—Theiler's mouse encephalomyelitis and Teschen disease of swine The recent description of Coxsackie virus has further widened the group It produces a clinical picture similar to poliomyelitis but, judging by its behaviour in mice, its primary attack is on muscle rather than the central nervous system

CLINICAL FEATURES

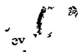
The incubation period is generally considered to be seven to fourteen days

The great majority of cases of poliomyelitis do not develop a frank paralysis Indeed, it is widely accepted that for every paralytic case there are 100 without paralysis The disease is therefore best described under three headings which are merely variable responses to infection As Burnet has pointed out, paralysis is only an incidental feature of attack by the virus

Abortive Poliomyelitis

In this there is a transient illness with fever, headache and pharyngitis Some cases show instead a gastro intestinal upset There is nothing typical clinically about the attack, and the symptoms are similar to those of many mild upper respiratory infections

The diagnosis of such cases is rarely, if ever, made except during epidemics Their importance lies in the fact that they are infectious for others



These cases sh[illegible] of the central nervous system [illegible] localizing signs such as paraly[illegible]

The disease begins with either upper respiratory or gastro-intestinal symptoms, fever and frequently headache Pain and stiffness in the neck and back are common, and if the head be drawn forward the patient complains of pain Kernig's sign is positive There may be slight head retraction, and when the patient is raised the head falls back Amoss' "tripod" sign can be elicited—a patient sitting up will place both hands behind him for support Paræsthesiae of the skin and excessive sweating frequently occur The patient may be irritable and restless, or apathetic and with ence phalitic symptoms

Lumbar puncture will show an increase in cell count at this stage The majority of cases do not progress further into the paralytic stage but make an uneventful recovery

The so called dromedary type of illness is stated to be of bad prognostic significance as a high proportion of such cases develop paralysis For two or three days the patient has fever, is drowsy, and often shows signs of upper respiratory infection The temperature then drops and the patient improves for as long, in some cases, as a week, when fever returns with signs of definite nervous involvement The first hump is said to correspond to the illness of infection, and the second to the beginning of the attack—the difference in time between them being the incubation period

DIFFERENTIAL DIAGNOSIS

Lymphocytic choriomeningitis 'serous' meningitis and respiratory tract infections may have to be outruled by the appropriate laboratory tests

Paralytic Poliomyelitis

There are several varieties described of which the commonest are the spinal polio-encephalitic and bulbar types There is practically always a history of illness, as described above, which may have been quite mild in many cases, but an occasional case may present without any previous notice able illness During the course of a pre paralytic illness

muscular twitchings and pain often point to the site of forthcoming paralysis, and there may be increase in muscle tone with an exaggeration of reflexes

Spinal Type

Fever subsides with the onset of paralysis which is of the flaccid lower motor neurone type with lost or diminished tendon reflexes Some muscle groups will be *hypertonic* because part of the reflex arc may be intact and continue to transmit impulses to them

It is customary to speak of the *involvement of groups* of muscles, but this is due to the action of the virus on their innervation There is no evidence that poliomyelitis virus acts directly on muscle

Paralysis is at its peak in five to seven days after which improvement sets in, often with great rapidity The muscles *most frequently affected are those of the lower limbs* particularly below the knee, and nearly all cases have leg paralysis with or without some degree of involvement of the arm or *trunk muscles* Isolated *paralysis of the arm* muscles is uncommon When it occurs the muscles of the shoulder girdle are usually selected

The immediate prognosis in this type of poliomyelitis depends on the muscles affected Involvement of the diaphragm, the intercostals or neck muscles is a bad prog *nostic sign* *Death frequently follows* from respiratory paralysis

CEREBROSPINAL FLUID

Changes occur early in the pre paralytic stage, the first being an increase in the cell count This is rarely high *the average figure* being under 100 cells per c mm The cells are lymphocytes but an equal proportion of polymorphs may be present for the first three or four days after which *they* decline in number During the third week the cell count begins to fall The total protein is also raised, usually rising later than the increase in cells and remaining *abnormal for*

longer It is highest during the third week In about half of all cases tests for globulin are positive Cerebrospinal sugar and chloride levels remain normal

Only one worker has claimed success in the isolation of poliomyelitis virus from cerebrospinal fluid

Polio-Encephalitic Type

Convulsions and cerebral manifestations occur in conjunction with paralysis

Bulbar Type

Paralysis affects the cranial nerves sometimes with involvement of the limbs or trunk (bulbo spinal type) The prognosis of bulbar paralysis varies depending on which area of the brain stem is involved Should there be signs of paralysis of the muscles supplied by the third fifth and sixth nerves or difficulties in mastication the closest watch should be kept for signs of further spread to the vital centres If this does not occur the prognosis is good

Involvement of the ninth tenth and twelfth nerves as shown by difficulty in swallowing or change of voice is a much more serious matter If the patient can be kept alive the prognosis is good but as will be seen energetic intervention including the use of mechanical respirators and tracheotomy may be necessary Once the vital respiratory and cardiovascular centres are affected the prognosis is almost hopeless should the latter be damaged death is inevitable

Other varieties of poliomyelitis have been described such as cerebellar meningitic and polyneuritic

MORTALITY

The reported mortality figures vary widely in different epidemics They depend on the one hand on the proportion of bulbar cases and on the other on the number of non paralytic cases reported Almost all deaths occur during the first week and hardly any after the second In the great

majority of cases death is due to respiratory paralysi anoxia

The mortality in bulbar cases is very high, ranging 60 to 80 per cent In all cases the prognosis for life is in children under one year and in the elderly

Taking an overall figure one may expect to find 75 per of cases in any epidemic making a good recovery In b cases those few who survive frequently show com recovery

PATHOLOGY

The virus may use any one of several routes of entry to central nervous system, as discussed below, but its at is confined to anterior horn cells and certain other neu in the cerebrum and midbrain No lesions caused by direct action of virus can be found outside the central nerv system, and even within it the attack is highly specific in location Certain areas, for example the occipital lobe, quite resistant to the virus, and often on direct experime inoculation show no lesions other than trauma due to inoculum

The action of the virus is on susceptible neurons only, all lesions in the cord or brain are due to the direct actio the virus on these cells Although there may be a considera degree of interstitial change such as perivascular cuffing meningitis in post mortem material, experimental work shown conclusively that the nerve cell damage is the prim change

To the naked eye the brain and cord are congested at po mortem On section the cord shows œdema with a sh demarcation between the grey and white matter

The microscopical picture is one of neuronal destructi exciting an inflammatory reaction in its immediate nei bourhood characterized by phagocytosis of dead ce (neuronophagia) by macrophages Polymorphs do not pl an important part in the process Neighbouring vessels sho

perivascular cuffing Inclusion bodies may be seen in the nuclei of cells attacked but not destroyed, but these inclusions are not in any way diagnostic of poliomyelitis

Distribution of Lesions

Lesions are found throughout the cord, principally in the anterior columns, but a few occur in the posterior and intermediate columns In the brain they show a typical distribution being found in the medulla, cerebellum, mid brain, basal ganglia and globus pallidus The cortex shows little, the few signs of involvement being confined exclusively to the motor and pre motor areas An important point is that changes are rarely found in the olfactory lobes

Little is known of the distribution of lesions in preparalytic or nonparalytic cases but experimentally they are known to be widespread in monkeys, so that the virus may be present in the central nervous system without producing paralysis The reason for the lack of clinical signs of paralysis is that, while there is a widespread reaction no one nerve unit supplying a muscle group is sufficiently damaged to cease functioning In all cases—paralytic or nonparalytic—microscopical changes are more widespread than would be expected from the clinical findings

Judging by the microscopical picture the primary action of the virus is on the cytoplasm of the cell and during recovery cells are found showing dissolution of the Nissl substance together with the intranuclear inclusions already mentioned Such cells are numerous during the first month but are not found later This month is the period of recovery in the central nervous system Any damaged cells which are going to regain their function have done so by the end of this time, and no further improvement in the nervous system can be expected

No lesions attributable to the direct action of virus are found in the muscles Muscular changes are secondary to nervous damage

Distribution of Virus

During and after an attack of poliomyelitis the virus is to be found in three situations First and most important for the individual patient is the high titre of it present in the central nervous system, which falls rapidly with the onset of paralysis and may be too low three weeks later to infect monkeys on inoculation It is present there also in the pre paralytic stage and in non paralytic cases

Secondly, and of epidemiological importance is its presence in the nasopharynx where it may be found for a few days before and after the onset of paralysis

Thirdly, and again important epidemiologically, virus can be isolated constantly from the fæces for more than a week after onset, and 50 per cent of cases will be excreting it at the end of a fortnight

DIAGNOSIS

The only certain method of diagnosis is the isolation of virus by the inoculation of susceptible animals with material from patients In fatal cases the cord should be sent to the laboratory, in others the fæces Cerebrospinal fluid blood or urine are useless for inoculation purposes Monkeys are inoculated intracerebrally or intranasally with prepared cord or fæcal suspensions

In most countries except the United States only one laboratory will be in a position to undertake the isolation of poliomyelitis virus as at present the only experimental animals which can be used for this purpose are monkeys or chimpanzees

RECOVERY

This begins early and improvement in the degree of paralysis is often obvious at the end of a week as cells attacked by the virus but not destroyed regain their function After a month improvement proceeds more slowly These later

changes are due, not to anatomical improvement in the cord, but to functional rearrangements and to the hypertrophy of unaffected muscles Recovery of muscle function is complete by the end of six months when no further improvement may be expected

PRINCIPLES OF TREATMENT

It would be futile in a work such as this to attempt the consideration of the modern treatment of poliomyelitis in any detail It will rarely fall to the lot of the general practitioner to treat a case throughout the illness Nor would he be wise, except in very exceptional circumstances, to attempt to do so On the other hand there would seem to be no reason why cases of poliomyelitis should not be treated in a general hospital, provided reasonable precautions are taken to prevent the spread of infection When it is appreciated that for one paralytic case there are one hundred non paralytic, it is clear that, at least during an epidemic, most of the other cases in the hospital will have been already infected with the virus Many hospitals, however, will not admit cases of poliomyelitis This is unfortunate, because in the acute stage of the disease the help of several specialists, who are only to be readily found in a large hospital may be required

None the less it is becoming clearer that the practitioner who sees the patient in the acute stage may have the ultimate prognosis in his hands Russell's findings as to the need for complete rest at this time have gained wide acceptance, and they now form the basis of the early treatment of an attack From his work and that of others, it is unquestionable that exercise in the febrile period leads to more severe paralysis later During an epidemic of poliomyelitis any febrile disease, particularly in a child should be treated by strict rest in bed If the case is one of poliomyelitis much subsequent suffering will have been averted, if it is not no harm will have been done Avoidance of exercise then may even make the difference between a paralytic and a non paralytic attack

Bulbar Paralysis

Every case in the early stages must be closely watched for evidence of further spread of the damage in the central nervous system and, above all, for signs of bulbar paralysis Should there be indications of paralysis of the ninth, tenth or twelfth cranial nerves the prognosis immediately becomes graver, and energetic steps must be taken to minimize the strain on the respiratory and cardiovascular centres

The onset and spread of bulbar paralysis are characteristically rapid, but if the patient can be tided over the acute phase the ultimate prognosis is good There may, however, be only a few hours to take those steps which make the difference between death and survival Any signs of respiratory embarrassment must therefore be promptly dealt with It is very probable that even minor degrees of anoxia would affect the vital centres thereby rendering them more liable to attack by the virus

To maintain an adequate airway it may be necessary to employ postural drainage, suction, mechanical respirators, or even, in the last resort, tracheotomy which may be the only means of saving the patient's life The indications for it are signs of difficulty in swallowing and a nasal voice Anoxia may be present to a dangerous degree even in the absence of cyanosis, and may be recognized by an increase in the pulse rate, restlessness and headache

Cheyne Stokes breathing is the sign of damage to the respiratory centre, and with its appearance the prognosis becomes correspondingly worse The final stage is involvement of the cardiovascular system when death rapidly supervenes

Spinal Paralysis

In uncomplicated spinal cases treatment by passive movement must be instituted as soon as possible after the acute stage, when there is no danger of any further spread of the paralysis It is usually undertaken about forty eight hours later This is the period of pain and muscle spasm Muscular

deformities, difficult or impossible to relieve later, supervene unless the patient is closely watched at this time For the relief of pain and spasm moist hot packs or radiant heat should be liberally applied Immersion baths are useful because they make it easier to start the passive movements

From this time forward treatment is obviously the province of the specialist in physical medicine The object is to utilize all the remaining nerve cells and to re educate the muscles by keeping them employed With modern methods the prognosis may be regarded as good for those muscles which show anything more than a trace of activity after the acute stage The end result to be aimed at, and it may take many months of treatment, is to restore the patient as nearly as possible to his condition before the attack The psychological background must also be kept in mind Any form of vocational therapy particularly in young adults, should take the form of training for a career rather than the "arts and crafts" methods so often used

EPIDEMIOLOGY

The modern history of poliomyelitis demonstrates strikingly the evolution of a disease Until quite recent years it was a sporadic endemic disease in all countries In a few it has now changed to an epidemic one while remaining endemic in others The change is illustrated by an examination of the age groups attacked by it Where the condition is endemic, and during the first big epidemic occurring in any country, the infantile age group is the one affected Up to 90 per cent of the cases occur in children under five years This happened in the first epidemics in Sweden Australia and the United States In the United States there has been a marked change over the past twenty years with relatively fewer cases in the 1 5 age group than in the 5 10 years group and an increasing number of cases is occurring in school children and young adults In 1939 41 only 36 per cent of cases were in the 0–4 years group In Massachusetts in 1907 about 7 per cent of

cases were over 15, in 1945 this proportion had *risen to 25 per cent* Such a picture is generally encountered today *in those* countries in which the standards of living and of *sanitation* are high Epidemics arise every few years with a higher proportion of cases in the older age groups

In other countries in which living conditions are less advanced, the original picture persists of small epidemics at infrequent intervals with a high incidence in the 0–5 age group *It is in those countries that the* condition is "Infantile Paralysis" Where the virus is endemic with these epidemic outcrops, the virus is freely dispersed in the environment and the bulk of the population has become immunized by about five years of age Hence the susceptibles are in the under five years group In advanced countries children are less exposed to virus There is, therefore, less immunization and there are larger pools of susceptibles, and in consqeuence, major epidemics with the main incidence falling on the school child

This conception of the epidemiology of poliomyelitis, as a disease picture being constantly modified by lack of child hood immunization due to improvements in living standards is supported by other evidence

It has long been recognized that there is a difference between urban and rural areas both in the incidence and the age dis tribution of cases There is a higher incidence in country districts and rural cases are older on the average than those in cities If resistance follows immunization by subinfective doses, it would naturally be lower in those districts where as human contacts are fewer, the virus would be less prevalent Similarly, it is in the country districts where childhood immunization is less frequent that one would expect the disease to attack older age groups than it does in the towns

Again, servicemen during the war often showed a higher attack rate overseas than did comparable groups at home These men were apparently attacked by a '*foreign*' strain of virus, and one to which they had not built up any immunity by exposure to subinfective doses Alternatively, of course

they may not have had sufficient resistance to overcome massive doses of virus present in high concentration in the environment

Geography

Poliomyelitis occurs in all climates as an endemic infection with an epidemic incidence principally in North America, Scandinavia, Australia and of late years in Great Britain It has recently been shown to occur inside the Arctic Circle

Seasonal Incidence

The peak incidence of the disease is usually during the summer and autumn, but little reliance can be placed on the virus obeying any rules Some outbreaks have continued into the winter before dying away, and others have begun in the winter months This latter, however, is uncommon Excessively heavy rainfall may be of significance, not only in the case of droplet infection, but also by increasing the possibility of fæcal contamination of rural water supplies such as shallow wells

Spread of the Virus

The virus is present in the nasopharynx of the patient, whether the infection is abortive or paralytic, for about three days after onset and infected persons may spread it by droplets Until some ten years ago it was thought that this was the only means of spread—a theory based on two well established findings Firstly virus was known to be present in the nasopharynx and secondly nasopharyngeal symptoms are common in the prodromal phase In some epidemics droplet spread seemed the most likely route, but there have always been difficulties in accepting it for all cases The usual seasonal incidence of the disease is quite unlike that of other droplet infections and in addition institutional and school outbreaks have always been uncommon

The presence of virus in the fæces was first proved nearly forty years ago, but was considered of little importance as

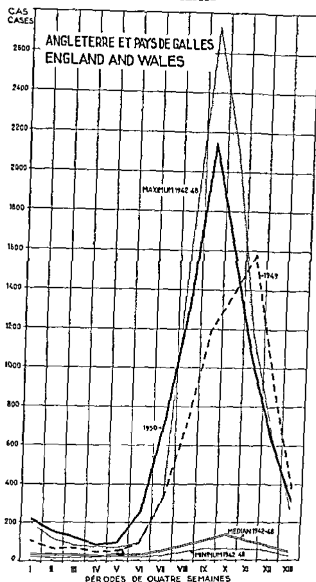


FIG 6 Cases of poliomyelitis reported in England and Wales by four week periods, 1942 1950

(By courtesy of the World Health Organization)

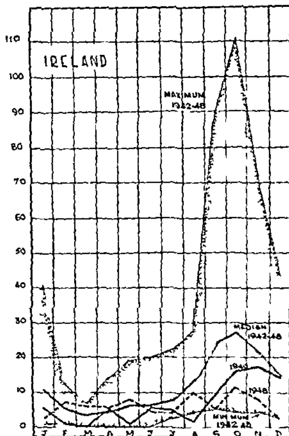


FIG. — Cases of poliomyelitis reported in Ireland by months, 1942-1949. Note that the median level for December is higher than that for August.

being merely the reappearance of virus swallowed from the nasopharynx When the significance of virus in the fæces was understood, its presence there for several weeks after infection, in some cases for months, led to the current and apparently well founded theory that fæcal spread is the most important single method of dissemination The fæcal material may be ingested with food contaminated by flies or washed in contaminated water, but more often it is trans ferred by contact with the excretor, or by fomites such as towels or eating utensils The virus is usually spread to close contacts particularly the family and, in children, the play mates Direct and sustained contact is necessary for infection, but it has been shown on many occasions that cases distribute the virus widely in their environment It has become increasingly common to find that infection can be traced to close contact with a previous case, abortive or paralytic, in the infectious period

Carriers

Carriers are unquestionably of importance in the spread of the disease, although in many cases it is difficult to track down the carrier responsible for any particular outbreak Such a search is more likely to be successful in rural areas

Convalescent carriers are of little significance as few cases excrete virus for more than one or two months in the fæces, or more than a few days from the naso pharynx Even should they be no longer in hospital precautions to prevent their infecting others can readily be taken

Healthy carriers, or those who contract infection but show no clinical evidence of it, excrete virus for similar periods and are obviously a formidable epidemiological problem

It is very probable that the St Helena epidemic of 1945–46 in which 215 cases were reported, was due to the visit of a healthy carrier

"Incubating" or "precocious" carriers excrete virus for several days before displaying symptoms, and have been known to infect others

Sewage

The virus has been isolated from the sewage even of large [illegible]

of water supplies and bathing pools The virus is relatively resistant to chlorine so that bacteriologically pure water is not necessarily free from the virus Privies are an even more important source of contamination particularly for flies, unless strict precautions are enforced

Flies

Although there is no evidence that the virus can multiply in flies, they may be very dangerous as merely mechanical carriers of infected material It has been shown experimentally that food exposed to the flies in an infected household was capable of infecting chimpanzees when fed to them There is no evidence to incriminate mosquitoes or biting insects

Milk

The virus can survive in milk for a period of some weeks and occasional outbreaks have been traced to contaminated milk As might be expected most of these were of an explosive nature

Food

Uncooked fruit or vegetables may be infected, particularly in Eastern countries

Reservoir

There is no reason to suspect that domestic or other animals may act as reservoirs of infection Even rodents, which are susceptible to virus infections closely related to poliomyelitis, do not appear to be of any significance

The virus survives between epidemics by continuing transfer in children who are themselves easily infected, and

who readily infect others by slight fæcal contamination of fingers with *transference to toys and other objects*

CONTROL

Patients are, as a rule, isolated for three weeks after onset, but as stated above a high proportion will still be actively excreting virus at the end of that time All fæces should be sterilized, and in the early stages also handkerchiefs and bed linen

Contacts

CHILDREN These should be quarantined for three weeks and arrangements made for the sterilization of handkerchiefs and fæces Although this quarantine may be carried out at home it should be kept as rigid as possible

ADULTS It is obviously difficult to isolate adults for any long period but those in contact with children, or engaged in handling food or milk should nevertheless be quarantined A reasonable scheme for adult contacts would seem to be quarantine for one week and restriction of movement for another two

The strictness of the measures which can be enforced is obviously dependent on the type of community involved Isolated rural houses or communities will be very much easier *to control than households in cities or towns*

Schools

In day schools in urban areas little purpose would be served by closure of the schools, although such a measure would be valuable in country districts if accompanied by instructions *to the children not to mix*

Residential schools should be quarantined, and if parents wish to remove children they should only be permitted to *do so if isolation en route* and at home for three weeks can be enforced In the unlikely event of secondary cases occurring

in the school the children may be sent home for not less than three weeks

During an epidemic every effort should be made to keep foodstuffs and milk covered Non chlorinated water supplies for drinking and domestic use should be boiled Fæces of cases and contacts should be sterilized and flies prevented from gaining access to them by proper disposal of sewage In privies, fæces should be rapidly and completely covered with earth D D T or other similar preparation should be used liberally, and breeding places of flies such as dung heaps in country districts should receive special attention Houses should be disinfected at the end of isolation periods Excessive exercise by children and bathing in pools, or even swimming baths should be forbidden

All operations such as tonsillectomies or tooth extractions should be suspended during an epidemic

PATHOGENESIS

It is considered at present that poliomyelitis virus is essentially neurotropic, reaching the central nervous system by nerve pathways from its point of entry The blood, cerebrospinal fluid and lymph have been suggested as vehicles for the spread of virus within the body, but without, so far at least, any satisfactory evidence in their favour Pathological findings do not suggest that any of them can be of importance, but there is no doubt that widespread changes in lymphoid structures such as the enlargement of glands and tonsils (which may contain virus) are found during an attack

Our present views on the entry of the virus into the nervous system have been influenced more by experimental work than by observation in man and in at least one respect this has led us astray in the past Until some ten years ago there was virtual agreement that the virus passed into the central nervous system by the olfactory nerves The problem has since been shown to be more complicated and there is now

little of the unanimity which previously prevailed The reason for the earlier view was that the most widely used experimental animal was the rhesus monkey, in which the natural route to the central nervous system is by the olfactory mucous membrane and the olfactory nerves In man, and in the chimpanzee, this route is also available but is not the natural route and is probably rarely, if ever, used by the virus

Before detailing the routes whereby the virus may reach the central nervous system several general observations may be made —

First, experimental work shows that the virus spreads by axonal routes from the site of inoculation and it seems unlikely that, except after trauma, any nerve without a superficial connection transmits virus to the central nervous system, not because it is unable to do so, but because it does not get an opportunity Common exceptions to this are the motor nerves in the pharynx after tonsillectomy

Virus entering the body first invades the superficial axons of a nerve cell, and passes along its fibres to the neuron Here it multiplies and travels on, the first synapse probably presenting a temporary barrier The position of the first neuron and synapse may vary considerably in their location according to the nerve involved In the parasympathetic system, for instance, they are encountered almost immediately beside the mucous membrane, while in the mesencephalic trigeminal they are in the midbrain—that is already within the central nervous system

Secondly the virus is not merely neurotropic, in having an affinity for nervous tissue, but is *neuronotropic* It is, therefore, dependent on the nerve itself rather than on any mesodermo glial elements of the nervous tissue The mechanism by which the virus travels in peripheral nerves well illustrates this point The virus combines only with the protoplasm of the axis cylinders and migrates along the axons themselves Perineural lymphatics or lymph spaces are of no importance in its passage nor does it travel between the various sheaths of the axon The actual mechanism of

transport within the axon remains unknown There is probably no multiplication there, the virus must reach the nerve cell body before propagation and further spread can occur

Thirdly, the virus will only travel along peripheral nerves which are both anatomically and physiologically intact On the other hand, its capacity for travelling within the nervous system is very considerable, and it may travel in either direction along a nerve

Finally as has been seen, poliomyelitis shows a specific attack on anterior horn cells and to a lesser extent, neurons in the midbrain and certain areas of the cerebrum

One of the difficulties in attempting to track down the path of the virus by the histological examination of nerves, is that when a neuron is found showing signs of invasion, whether in bulb, brain or ganglion it is impossible to say in which direction the virus was travelling at the time of invasion Once the virus has entered the central nervous system it spreads widely throughout and an affected ganglion may have been invaded during the later, centrifugal spread of infection rather than in the primary invasion

Having discussed the general principles of infection one may now consider briefly the entry of virus into the body Various routes may be implicated

The Nose

As has already been discussed there are many features of the disease in favour of entry through this area to which may be added the fact that the olfactory nerve endings lie exposed in the nasal cavity All of these add up to impressive evidence in favour of the nose as an important portal of entry

Against the olfactory nerves are two factors In the first place, histological evidence derived from fatal cases in man shows that lesions rarely occur in the olfactory bulb Oddly enough relatively little work has been done on this aspect of infection in spite of the importance allotted it up to some ten years ago

The second piece of evidence against the olfactory nerves being of any importance is the complete failure of various chemical sprays such as zinc sulphate and picric acid designed to block the nerve endings In field experiments they proved of no value in prophylaxis

To sum up while virus may enter the *body* by the nose and naso pharynx it does not reach the central nervous system by means of the olfactory nerves It may however enter by means of the cranial nerves in the nasopharynx which will now be considered

Mouth and Pharynx

Assuming as one must spread by either infected droplets or fæcal contamination it is obvious that virus enters the body in this region In addition it has frequently been isolated from tonsils and naso pharyngeal secretions in all types of cases paralytic or abortive and in healthy contacts When to this is added the frequency of bulbar paralysis following on tonsillectomy the closest attention must obviously be paid to this area Histological evidence in this case is unequivocal that virus may spread to the central nervous system by the afferent systems of the fifth ninth and tenth cranial nerves which may be infected by the slow drainage of nasal mucus containing virus or by material entering the mouth

Tracheo Bronchial Tree

This has also been implicated the nerves concerned being the ninth and tenth efferent systems of the sympathetics

Stomach and Small Intestine

The ease with which virus can be isolated in the *fæces* the gastro intestinal symptoms which may occur in the pre-paralytic stage and the lymphatic lesions for example in Peyer s patches all point to the possibility of the virus entering by this route It is more than possible that polio myelitis virus may *multiply somewhere in* the wall of the gastro intestinal tract where or in what cells is unknown

because it is difficult to assume that all the virus found in the fæces must necessarily have been swallowed by the patient As little as 10 mgm of a wet specimen has been found infective for monkeys when inoculated intracerebrally The nerves involved would be the cranial nerves or the vagus leading to the medulla or, alternatively, visceral afferent or efferent fibres of the sympathetic chain leading to the spinal cord

Colon and Rectum

These are of no significance as portals of entry

In spite of the convincing evidence of the presence of virus in the intestine it seems most likely that the virus more usually enters by the upper alimentary route There is no exclusive route of entry to which one may point as the earlier workers pointed to the olfactory nerves but the most important channels are probably the oropharynx and the nasopharynx

The *region of initial involvement of the central nervous* system does not necessarily determine the level of initial paralysis Once introduced into the central nervous system which may happen almost simultaneously with its introduction into the body, the virus spreads rapidly throughout it Even then there is no reason to suppose that paralysis must necessarily follow Violent exercise followed by almost immediate paralysis of a limb is a commonplace *during* epidemics Poliomyelitis virus may therefore apparently lie dormant in the nervous system until such time as the exercise or some other mechanism upsets a pre-existing and silent relationship between the host cell and the virus and gives rise to paralysis

IMMUNITY

The problem of immunity in poliomyelitis is a difficult one There is every reason to suppose that the virus can elicit antibodies as happens in other virus diseases Similarly,

there is no reason to suppose that such antibodies are produced by any other means than by contact with poliomyelitis virus The problem lies in determining what part such antibodies *may play in resistance to infection*

It is generally thought now, on the basis of experimental work in monkeys that resistance to infection is determined by some alteration in the nervous tissues rather than by circulating antibodies in the blood It is not in dispute that contact with subinfective doses of virus builds up the immunity which is found in most adults, but it seems that this immunity is based not on humoral antibodies but on the insusceptibility of the nerve cell which follows

As the virus is present in the central nervous system from the beginning, and there is no stage of primary spread in the blood it would seem that antibodies present in the blood can be regarded as incidental to the course of infection whatever form, paralytic or otherwise, the latter may take Some virus escapes into the blood thus giving rise to anti bodies These are of no practical significance They merely show that the virus has been present in the body and has attacked *some nerve cells*

How the mechanism of immunity in the nervous system works is unknown It has been attributed to the lymphocytes present in and around *the damaged areas and to the microglia* which may be part of the reticulo endothelial system and even more numerous in the lesions than the lymphocytes Alternatively some change in susceptible cells *rendering* them refractory to infection, may be produced by the clinically unrecognizable entry of the virus

It follows that any form of therapy using immune serum should be of little value, and so it has proved in practice Serum therapy presupposes a stage of the infection in which the virus would be susceptible to attack By the modern theory of the pathogenesis of poliomyelitis the virus is present in the central nervous system from a very early stage, and any virus present in the blood at any stage is there by accident, as it were Even if the immune serum had any

action on the virus it is difficult to see how it can come in contact with it, for the antibody cannot enter any body cell, nor any axon, to exert its protective action

Active immunity following the use of vaccines has had an extensive trial in the United States Brodie's formolized vaccine showed equivocal results in that in its largest field trial none of the controls developed poliomyelitis, and so the efficacy of the vaccine could not be assessed Doubts have been expressed as to whether the preparation can be regarded as completely avirulent Kolmer's ricinoleated vaccine is known to contain active virus, and in a very extensive trial, although none of the children who received the full course of three doses developed poliomyelitis, there were ten cases amongst those receiving fewer doses After this unfortunate occurrence the vaccine was withdrawn, and it seems unlikely that any vaccine containing virulent virus will again be used

PROPERTIES OF THE VIRUS

The properties of the virus are not as well known as those of many others This is due not only to its very small size but also to the difficulty of cultivation Poliomyelitis virus

obtaining pure suspensions of virus free from extraneous matter electron microscopy has been difficult The Lansing strain has been shown to be composed of spherical or slightly asymmetrical particles some 25 mμ in diameter

No satisfactory growth has been obtained with tissue culture or in the chick embryo Although some reports have claimed that the Lansing strain has been propagated in the chick embryo the work awaits further confirmation Recently it has been reported that the virus will multiply in tissue culture if human tissues are used

Poliomyelitis virus is relatively resistant to chemical agents It is extremely resistant to glycerol retaining its

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PROPERTIES OF THE VIRUS

The properties of the virus are not as well known as those of many others This is due not only to its very small size but also to the difficulty of cultivation Poliomyelitis virus is one of the smallest known pathogens of man Ultrafiltration and centrifugation studies have shown that it measures about 15–25 mμ in one diameter, but due to the difficulty of obtaining pure suspensions of virus, free from extraneous matter, electron microscopy has been difficult The Lansing strain has been shown to be composed of spherical or slightly asymmetrical particles some 25 mμ in diameter

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Poliomyelitis virus is relatively resistant to chemical agents It is extremely resistant to glycerol retaining its

activity in a 30–50 per cent solution for a period of years It is difficult to assess the value of chlorine in inactivating it in public water supplies, as according to some workers at least five parts per million of chlorine—a figure in excess of that which will give a palatable water supply—have been insufficient Others have found that a very much smaller amount is sufficient to inactivate the Lansing strain for example As the sterilization of water supplies does not depend on the action of chlorine alone, it is probable that about 0 1 to 0 2 parts per million will suffice While the virus can survive in water for very long periods there is little evidence that public water supplies, as distinct from shallow private wells, are ever involved in the spread of infection

A temperature of 55°C destroys the virus in thirty minutes, but it may survive at —20°C or —70°C for periods up to twelve months

It is quite insusceptible to chemotherapy

THE COXSACKIE GROUP OF VIRUSES

Within the past two years a new group of viruses, which produce a clinical picture very similar to that of poliomyelitis, has been described Coxsackie virus was first isolated from two children in the village of that name in New York State during a mild outbreak of what was thought, on clinical grounds, to be poliomyelitis Since that time a number of other strains of the same agent have been described and it is clear that there are several strains comprising the group

The existence of such a virus was first suspected when it was noticed in 1947 that there was a marked discrepancy in some outbreaks between the notifications of paralytic and non paralytic poliomyelitis In the same summer an outbreak of what appeared to be poliomyelitis occurred in Delaware, which was unusual in having a remarkably low mortality Coxsackie virus was also isolated from some of these cases

Not alone does the disease caused by Coxsackie virus closely resemble poliomyelitis clinically, but the two viruses have, on occasion, been recovered from the same patient. The virus has been isolated from cases of paralysis, some of which showed residual paralysis, but it is still not clear if it can cause paralysis in man. It has not yet been possible to outrule the possibility of concurrent infection with poliomyelitis in these cases.

Clinical Features

So far as is known at present, infection with Coxsackie virus may produce any one of three clinical patterns: "non paralytic poliomyelitis", epidemic myalgia (Bornholm disease, pleurodynia), or a minor undifferentiated febrile illness. The first group is clinically indistinguishable from true non paralytic poliomyelitis, and in some cases the illness has even been diphasic. In those in which poliomyelitis could be definitely outruled the illness was short, lasting at most two weeks, and the patients made complete recoveries. In the second group the clinical signs and the results of physical examination are similar, but the patient complains of severe abdominal or thoracic pain which may persist for about two weeks.

Clinical laboratory examinations are of little assistance in the diagnosis of cases. There is generally a slight increase in the cell count in the cerebrospinal fluid, but the protein appears to remain within normal limits. None of the other routine findings are of significance.

Little is known of the pathological features of the disease in man. In infant mice and hamsters the virus produces paralysis, but its primary effect is on the muscles rather than on the nervous system. In some animals lesions have been described in the cerebral hemispheres but the anterior horn cells have never been found to be affected. The muscle lesions in mice are those of a severe myositis which is especially marked in the limbs. In some areas a hyaline necrosis resembling Zenker's degeneration is found.

Epidemiology

The epidemiology of Coxsackie virus appears to be so similar to that of poliomyelitis that nothing would be gained by going into any detail concerning it The virus is present in the nasopharynx and in the fæces where it may persist for some time In one case it has been isolated from the stools *73 days after the onset of symptoms* It has also been recovered from flies and from sewage during epidemics sometimes in association with poliomyelitis Healthy carriers of Coxsackie virus have been described and family outbreaks have also occurred

One point of distinction appears to be in the age distribution of the two viruses Coxsackie seems to affect predominantly children under the age of ten years In the United States, with its increasingly older age distribution of poliomyelitis, this may mark a significant difference

Most of the field work on Coxsackie virus has been carried *out at times and in places where poliomyelitis was epidemic,* so that there seems to be a very close relationship with the latter How close this relationship actually is remains to be seen for it may well be that a different picture would be presented by investigations undertaken when poliomyelitis was not active In other words some differences in the epidemiology of the two conditions may become apparent in the future

Properties of the Virus

The outstanding characteristic of the Coxsackie viruses is their laboratory predilection for suckling mice and hamsters On occasion lesions have been produced in older rodents, but for the primary isolation and subsequent propagation of the virus infant animals are required The best results are obtained by using them when less than twenty four hours old *Cynomolgus monkeys* and chimpanzees may also be infected but no clinical signs of the disease can be elicited

The virus is destroyed in thirty minutes at 60°C, but will survive for long periods at —20°C or —70°C It is resistant

to glycerol in which infectivity can be preserved for two months It is also unaffected by ether

The particle size has been estimated as between 15 and 23 mμ and the virus is readily filtrable

Several immunological types of the virus occur and there seems to be relatively little antigenic overlapping between them Whether this finding is of importance in the epidemiology of the disease is unknown

POLIOMYELITIS AND PROPHYLACTIC INOCULATIONS

During 1948–1949 two sets of observations one in the London borough of St Pancras and the other in Victoria Australia drew attention to the frequent occurrence of poliomyelitis in patients who had received a prophylactic inoculation within the preceding two months In all cases the paralysis occurred in the limb which had been injected At first sight this relationship appeared to be no more than coincidental particularly as poliomyelitis had been epidemic in the areas concerned Further investigations however, revealed that the association was statistically significant and that most of the cases arose within seven to eleven days after injection a time within the incubation period of poliomyelitis

These observations have been repeatedly confirmed and there seems now to be little doubt of a relationship between the paralysis and the inoculation The materials most often implicated have been pertussis vaccine and diptheria A P T, either combined or separately In the Australian cases pertussis vaccine appeared to play the major role but in England there was rather more evidence favouring A P T Cases have also been reported following T A B inoculation and penicillin injections so that it seems at present as if the material injected may not be of importance It may be the injection and not the inoculum which is the precipitating factor

It is difficult to explain these cases Contamination either of the material or of the needle seems to be out of court, as the cases occurred in widely scattered places and with different antigens In any event, in the absence of a viræmia in poliomyelitis, there seems little possibility of a needle being contaminated The most likely reason seems to be trauma at the site of the injection which affects the local nerve trunks in a patient in whom the virus is already present Much more work remains to be done to obtain the true explanation, but the one given seems the most reasonable of those suggested so far

One of the most interesting features of the condition is that no cases have as yet been described in the United States Whether or not differences in inoculation techniques may play an important part in the ætiology of these cases it is too early to say, but the lack of reported cases from America is very suggestive

Finally, one may remark that it would be a very serious matter if prophylactic inoculation against diphtheria should fall into disrepute because of these relatively few, cases of poliomyelitis following injection It is quite clear that in those communities in which immunization has been practised, diphtheria has declined to negligible proportions in recent years The major credit for this must go to prophylactic inoculation At its worst, diphtheria is a far more deadly disease than poliomyelitis and there should be no thought of suspending immunization except in the face of the most unequivocal evidence At present this is not available

Chapter Seventeen

MENINGITIS AND ENCEPHALITIS

MENINGITIS CAUSED BY VIRUSES

The clinical picture termed acute aseptic meningitis may appear during the course of infection with a number of viruses These include amongst others, measles, mumps, varicella and herpes, and the clinical and pathological features are described in the respective chapters dealing with these diseases In addition, the virus of lymphocytic chorio meningitis must be considered by itself as a primary cause of meningitis

LYMPHOCYTIC CHORIOMENINGITIS

This virus was unexpectedly recovered during the investiga tion of an outbreak of St Louis encephalitis and it has since been shown to be widespread amongst mice Subclinical attacks appear to be frequent in humans, at least in the United States Whether such findings are applicable to other countries has not yet been fully proved, but there seems little reason to doubt that they are

Clinical Features

After an incubation period variously estimated at one to three weeks the onset is acute, and the attack may be clinically very similar to one of influenza In many cases the patient recovers from a febrile illness in the course of a few days, no further sequelæ develop, and the attack is classified merely as "pyrexia of unknown origin"

Other cases show definite signs and symptoms of meningitis which come on after the influenzal phase and last about

a fortnight Headache, vomiting and a stiff neck are prominent features The cerebrospinal fluid is under pressure and shows a small increase in protein content The cells are increased, usually numbering about 200 per c mm but counts up to 2 000 per c mm have been reported They are, needless to say, lymphocytes Complete recovery ensues and there is rarely any permanent disability

Diagnosis

The final diagnosis rests exclusively on laboratory tests The virus may be isolated from the cerebrospinal fluid, the blood or the urine by the intracerebral inoculation of mice It is also present in the nasopharynx during the acute stage Care must be taken that the laboratory stock for inoculation is free from the virus before being used for such tests

Infection stimulates the production of complement fixing and virus neutralizing antibodies, and the increase in titre between an early and a late specimen of blood is diagnostic Complement fixing antibodies may be present by the end of the first week and can certainly be demonstrated three weeks after onset They disappear again very rapidly, however, and may not be found at the end of six weeks The virus neutralizing antibodies appear later and last longer It may take as long as ten weeks before they are demonstrable, but they persist for some three years after infection

Pathology

The disease is rarely fatal and little is known of the pathological features The few cases examined showed a widespread lymphocytic infiltration of the meninges and the choroid plexus

Epidemiology

As already mentioned the virus is common amongst mice, including domestic mice, and these appear to be the main source of human infection They excrete the virus in the urine fæces and semen, and man is probably infected by the inhalation of dust containing virus particles Mosquitoes and

ticks have also been incriminated but are of far less importance

Sporadic cases occur during the winter and spring months Most work on the disease has been done in the United States but it is probable that cases occur throughout the world

Properties of the Virus

The virus is resistant to 50 per cent glycerol for several months, and it can also be preserved for about one year by freezing and drying Monkeys mice and guinea pigs are susceptible to infection Whilst growth occurs on the chorio allantoic membrane of the chick embryo there are no specific lesions to be seen

VIRAL ENCEPHALITIDES

The various members of the arthropod borne encephalitides, which are now to be described, appear to have more in common than merely their method of transmission It is still too early to speak of them as a strictly defined "family," but several of them possess antigens which are shared by other members of the group For instance, serological tests have shown that the viruses of St Louis encephalitis and Japanese B encephalitis are related Similarly, Eastern and Western encephalomyelitis viruses share antigens in common Again, the two tick transmitted members louping ill and Russian spring summer encephalitis, are related No one antigen common to the whole group has yet been described but there would seem to be a distinct possibility that one may exist

Hammon to whom much of our knowledge of the group is due has carried out a most interesting series of experiments from which he suggests that the various members comprising the group may have a common ancestry He and his colleagues isolated a virus from mites which on passage through mice appeared to be St Louis virus, but on being passed through fertile eggs was serologically identical with the

Western equine virus It seems unlikely that the starting material was a mixture of the two viruses

In support of these findings is the fact that both viruses have been found in the same district at the same time, and that double infections—with the two viruses—have been described

It would seem that, as well as the occurrence of infection with the fully characterized virus, such a "stem" virus might also be capable of infecting man or animals, and that it might then depend on the host whether it "evolved" to the St Louis or the Western equine virus

The proof of the existence of a stem virus which is suggested by these experiments would be of extraordinary interest, not only epidemiologically but also in the field of virus genetics, and confirmation will be awaited with great interest If it were provided it would bring together many of the loose ends suggested by the serological relationships already demonstrated between

In the field of help con siderably in the problems of the virologist—the relationship between the virus in nature and in the laboratory

ST LOUIS ENCEPHALITIS

This condition, which has so far been reported only in the United States, first came to notice when an outbreak of encephalitis occurred in 1933 in St Louis and other cities The ætiology of the condition was demonstrated by the isolation of a virus from many of the cases and by serological investigations A similar outbreak in 1937 was caused by the same virus It is now widespread throughout the United States where it causes small outbreaks and sporadic cases of encephalitis

Clinical Features

The incubation period varies from one to three weeks There is a sudden onset with marked pyrexia which may reach

105°F within forty eight hours Neck rigidity is prominent and there is no doubt of the encephalitic involvement Kernig's sign is positive and altered reflexes are found Mental disorder of some degree is almost invariable but, in contrast to ence phalitis lethargica, ocular palsies are rare The temperature falls gradually to normal at the end of ten days and the patient's condition improves as it falls The over all mortality is about 20 per cent but it is significantly higher in the older age groups

In contrast to this picture a number of cases do not display a sudden onset, but have a prodromal period lasting up to four days during which there is malaise, headache and mus cular pain At the end of the prodromal period encephalitic symptoms supervene and the subsequent course of the disease is the same

On examination the cerebrospinal fluid shows only a slight increase in lymphocytes and protein A polymorphonuclear leucocytosis may be found in the blood but, as leucopenia is equally common the blood picture is of little value in diagnosis

Pathology

There is a more severe destruction of neurons than in encephalitis lethargica and the lesions tend to be more wide spread More particularly the cord is involved to a greater extent The blood vessels are engorged both in the meninges and throughout the brain Perivascular cuffing is marked and there is a widespread infiltration with lymphocytes and plasma cells The changes are most marked in the brain stem and in the cerebral cortex

Laboratory Diagnosis

The virus is not present in the cerebrospinal fluid at any stage of the disease and has only once been isolated from the blood Attempts at isolation are therefore necessarily confined to fatal cases Suspensions of brain and cord removed post mortem should be inoculated intracerebrally into mice

The autopsy must be carried out as soon as possible after death, and the earlier death occurs in the disease the more likely it is that attempts at isolation will be successful

In the survivors either virus neutralization or complement fixation tests may be carried out They are of little clinical value, but are of epidemiological importance and should be performed when possible Such tests may be the only means of differentiating an attack of St Louis encephalitis from other forms Paired sera should be examined—the first collected during the first week and the second about three or four weeks later Some authorities recommend the collection of three samples of blood, the first in the acute stage, the second two or three weeks after onset, and the third up to eight weeks after the beginning of the attack The reason for this is that neutralizing antibody appears at different times with the various encephalitic viruses In the western equine variety, for instance, it appears during the first few days, while with St Louis virus it may not be apparent until the third week By using three specimens one is therefore reasonably sure of making a diagnosis This is the more important because it is impossible to differentiate clinically between the virus encephalitides and outbreaks have occurred in which more than one of them was involved

Epidemiology

The disease displays a very definite seasonal incidence, cases only occurring in the summer and autumn months No age is exempt but there is a marked tendency for the older age groups to be attacked

The pattern of the early outbreaks pointed to an insect vector, a suspicion which later work confirmed From field investigations it was considered that mosquitoes were the most likely vectors and the virus has since been recovered from them on many occasions in epidemic areas The most important varieties appear to be *C tarsalis* and *C pipiens*

The animal reservoir of the disease has not yet been demonstrated with certainty but is thought to be in chickens and

birds It is almost certain that these are themselves infected by mites or ticks in which case the latter would constitute the real reservoir of the virus

Properties of the Virus

SIZE

Filtration experiments have shown that the particle size is between 20 mμ and 30 mμ and the virus passes easily through the usual filters

PHYSICAL AGENTS

Activity is rapidly lost at room temperature and even in the ordinary refrigerator, but the addition of 50 per cent glycerol preserves it for some time at 4°C It is kept best at —70°C

Inactivation is complete after heating for thirty minutes at 56°C

EXPERIMENTAL ANIMALS

Mice are readily infected on intracranial or intranasal inoculation Monkeys are also susceptible but not with such regularity

The virus grows well in tissue culture and also in the chick embryo On chorio allantoic inoculation of the latter the membrane becomes œdematous but no specific pocks are found The virus is present in the membrane and in most of the tissues of the embryo itself

Antigenically the virus of St Louis encephalitis is closely related to although not identical with Japanese B encephalitis

JAPANESE B ENCEPHALITIS

This condition is so called to distinguish it from encephalitis lethargica—encephalitis A of the Japanese workers—and it is widespread in the Far East

Clinical Features

The incubation period is unknown There is a well marked prodromal period lasting anything up to four days, which is characterized by headache, drowsiness and nausea This gives way to a high temperature and meningo encephalitic symptoms During the acute phase paralysis and signs of mental impairment are found The attack lasts ten days to two weeks, by the end of which time the temperature has returned to normal If death does not occur in the acute stage complete recovery with no sequelæ is the rule, but the mortality is high and may reach 80 per cent in the elderly A small proportion of the survivors may display subsequent parkinsonism

A polymorphonuclear leucocytosis is found in the blood in the acute stage, and the cerebrospinal fluid shows an increase of lymphocytes

Pathology

The most striking change found microscopically is the damage done to the Purkinje cells of the cerebellum There is also perivascular cuffing and degeneration of neurons principally in the basal nuclei, pons, medulla and cortex The meninges show an extensive infiltration with lymphocytes

Epidemiology

Japanese B encephalitis occurs in large epidemics during the summer months and thousands of cases may be reported in any one outbreak The disease is found over a large area —Japan Korea, China and the Far Eastern territories of the U S S R

Infection is spread by mosquitoes, from which virus has been isolated in epidemic times There is a reservoir of the disease amongst animals but it has not yet been finally identified Horses, dogs and cattle seem to be the most likely source The method of transmission in non epidemic times is unknown It has been suggested that ticks may play an important part in the survival of the virus

Diagnosis

As with the other encephalitides the virus can be isolated from the brain in fatal cases by the intracerebral inoculation of mice Serological tests may be used in the diagnosis of the disease in those who survive As mentioned in the section on St Louis encephalitis, two or three specimens of blood should be collected at the appropriate times

The antigenic relationship between Japanese B encephalitis and the St Louis virus has already been noted This is not a serious drawback for diagnostic purposes as the homologous titre is always higher than the heterologous

Properties of the Virus

The size of the virus is 20 mμ to 30 mμ

The most satisfactory experimental animal is the mouse, which is readily infected by intracerebral injection

The virus also grows well in the chick embryo After inoculation by the chorio allantoic or yolk sac routes the virus is harvested at the end of forty eight hours, when it is present to a high titre in the tissues of the embryo itself Incubation beyond forty-eight hours usually results in the death of the embryo

EQUINE ENCEPHALOMYELITIS

This condition is widespread in the western hemisphere where several varieties have been described which affect man. The principal viruses concerned are Eastern and Western equine encephalomyelitis in the United States and Canada, and Venezuelan encephalomyelitis

The eastern and western varieties are so called because originally they appeared to be confined to the Eastern and Western States of the United States respectively Within the past decade, however, the eastern virus has shown a tendency to spread, and both viruses now occur in some states Antigenically the two viruses are closely related, but the eastern variety causes a very much more severe illness

Large summer outbreaks of equine encephalomyelitis occur from time to time, during which numerous human cases are reported The clinical, and also the pathological, features of the human disease are almost indistinguishable from those of St Louis encephalitis, and the two conditions may even be present in the same locality at the same time As might be expected, it is a rural disease

The mortality with the western virus is in the neighbourhood of 10 to 15 per cent In cases caused by the eastern virus it is very much higher, being about 70 per cent, and sequelæ such as paralysis and mental impairment are found in a high proportion of the survivors In one outbreak in which the survivors were followed up after ten years it was found that of thirty four original cases only one was apparently completely recovered Twenty five of the cases died in the acute stage, and one of the other nine could not be traced Two of them were dead, having been mentally deficient since the attack The five others were also mentally deficient, four of them with associated hemiplegia

The diagnosis is made by the isolation of the virus from the brain of fatal cases or by serological tests The western virus has, on occasion, been recovered from the blood and the cerebrospinal fluid

Both the western and eastern viruses are spread to man and horses by mosquitoes The most important species incriminated seems to be *C tarsalis*, but virus has also been isolated from other varieties Once again, as with St Louis encephalitis, wild and domestic fowl are of significance as a reservoir and serological surveys have shown them to be frequently infected Man and horses are apparently only incidentally involved the normal cycle being mosquito—fowl—mosquito What part ticks or mites play in the spread of infection amongst chickens has not been finally determined, but the western virus has been isolated from mites which are capable of transovarian transmission of virus These may very well be the host in which the virus survives during the winter months

Efficient vaccines are now available for the protection of animals, but little is known of their value in man None the less, such protection as they elicit should be made available to those at risk, particularly those liable to be bitten by infected mosquitoes during an epidemic Simultaneously, every available means of mosquito control should be intensively carried out

LOUPING ILL

This infection of sheep is of some importance, because since 1948 several cases of infection in farmers and shepherds have been reported Another case was an abattoir worker in Glasgow, and neutralizing antibodies to the virus were present in the blood of three of his fellow workers These are the first cases reported of the natural transmission of the disease to man Previously a number of laboratory infections had been reported, but it had been considered that humans were never involved unless working experimentally with the virus

The condition is usually described as occurring amongst sheep only in Scotland and in Northern England It is also found in Ireland, where it is widespread, and the virus has recently been reported to have been isolated in Russia The virus is antigenically closely related to that of Russian spring summer encephalitis

Amongst sheep the virus causes an acute encephalomyelitis in which the cerebellum is particularly affected, leading to cerebellar ataxia and leaping (louping) movements In man there is characteristically a diphasic illness It begins with pyrexia and an "influenzal" illness lasting a few days before the temperature drops and the patient feels well again A week or so later the temperature again rises abruptly to about 102°F, the patient may complain of some headache and diplopia, and there are well marked signs of encephalitis After five days the temperature falls by crisis and the patient improves rapidly Sequelæ particularly ataxia, may persist for some time but apparently are not permanent

The virus is spread in nature by the tick, Ixodes ricinus and in the human cases reported as being infected in the field there was ample exposure to these ticks In one case it was reported that very large numbers of them were present on the patient's body on admission to hospital

RUSSIAN SPRING-SUMMER ENCEPHALITIS

Clinically and pathologically this condition resembles Japanese B encephalitis and St Louis encephalitis It has not yet been reported outside Russia where it occurs principally in the Far Eastern territories The disease is one of summer months and is tick borne, the highest incidence being amongst those working in virgin forest The mortality during the acute phase is about 30 per cent, and permanent paralyses are frequent amongst the survivors As already noted, the virus is antigenically very similar to that of louping ill, but it causes a much more severe illness than the latter when transmitted to sheep

Chapter Eighteen

VIRUS INFECTIONS OF THE EYE

Three virus infections specifically affecting the eye are of importance Two of them, trachoma and inclusion conjunctivitis give similar clinical pictures and the viruses which cause them are closely related The third, epidemic keratoconjunctivitis is the only one to have yet been isolated, but it bears no relationship to the others Apart from these it is well known that in other virus infections ocular involvement is more or less common during the course of the disease Examples of these are herpes simplex, herpes zoster, lymphogranuloma, measles and smallpox Several cases of conjunctivitis due to Newcastle disease of fowl have been reported but these have occurred only amongst laboratory workers dealing with the virus

TRACHOMA

Clinical Features

The classical description of the disease is that of McCallan who describes the clinical course under four headings —

Trachoma Stage I (Tr I)

The incubation period is difficult to estimate as the onset of the naturally occurring disease is insidious The first sign of infection is the formation of tiny, rounded follicles in the upper fornix Simultaneously with this there may be some transient clouding of vision In other cases these follicles are not seen, and the most striking feature is a red velvet like appearance of the conjunctiva This is the result of wide spread subepithelial infiltration with lymphocytes and chronic inflammatory cells

Tr. II (a)

In this second stage small blebs about the size of a millet seed are found These burst readily on pressure, and this procedure was, until recently, the classical method of treatment in the early stages

Tr II (b)

If there is marked secondary bacterial infection the picture is modified, and papillary hypertrophy is prominent

Tr III

This follows either of the above stages, and is characterized by partial scarring which compresses the islands of tracho matous conjunctival epithelium Involvement of the cornea is now evident, so called "pannus," which is shown by the vascularization of the cornea This is due to the formation of new blood vessels, and is a sequel to the sub epithelial infiltration of the early stages

Tr. IV

The end result of infection is the replacement of epithelium and the sub epithelial infiltrations by dense fibrous tissue which, by its contraction, leads to trichiasis and entropion

The characteristic features of trachoma are the pannus and the dense scarring In addition, the changes involve the upper half of the conjunctiva and the cornea to a much greater extent than the lower

Treatment

Treatment with sulphonamides has had an extensive trial and their clinical value is unquestioned Whether the effect is a primary one on the virus or is merely on the secondary bacterial invaders is still controversial The writer has, on several occasions, found the typical inclusion bodies in con junctival scrapings taken after an apparently adequate course of sulphonamide therapy Penicillin has not given such satisfactory results as the sulphonamides, and its efficacy is

doubtful Aureomycin, on the other hand has given dramatic clinical results in some cases

Epidemiology

The distribution of the disease is world wide and cases have been reported from practically every country In the Mediterranean area particularly Egypt and Palestine, and large parts of Asia, it is an almost universal infection It was widely disseminated throughout Europe by soldiers returning from Napoleon's expedition to Egypt It occurs in certain parts of the United States, particularly amongst the Indian population, and in Canada In England it is rare and in Ireland has almost entirely disappeared, only a very occasional case now being seen

While, as already noted, the incubation period of the natural disease is unknown in experimental work with human volunteers it has been found to be as short as four to seven days It is very probable of course, that a larger inoculum was used in these experiments than would be present in natural infection

Spread occurs from case to case by the transference of infected conjunctival secretions, and cases are on record of ophthalmologists being infected by trachomatous patients Amongst the poorer classes in warm countries flies appear to be important as mechanical carriers of infection

Immunity

An attack confers no permanent immunity either in man or in experimental animals

Ætiology

Three different agents have been suggested as the cause of trachoma—rickettsial bacterial and viral

Various workers have described the occurrence in conjunctival epithelial cells of rickettsiæ which were distinguishable from the inclusion bodies described below Successful isolation of these rickettsiæ in lice has also been claimed

This claim is not generally allowed, and the "rickettsiæ" seen microscopically have been dismissed by most as being merely epithelial debris in the conjunctival cells Investigations using the Weil Felix reaction have shown that it is positive no more frequently in trachomatous than in non trachomatous patients

Noguchi reported the isolation of a bacterium, which he named *B granulosis*, and for which he postulated a causative rule It is now considered to be no more than a secondary invader

The virus theory goes back as far as 1907 when Halberstadter and von Prowazcek described the occurrence of cytoplasmic inclusion bodies in conjunctival epithelial cells The inclusions were masses of minute elementary bodies each about **0·25** μ in size Larger, basophilic, cocco bacillary bodies called initial bodies about 1 μ in length were later described by Lindner These large and small particles were suggested to be the causal agent, and Thygeson has stated that they are stages in the life cycle of the virus According to him it undergoes a regular sequence of morphological variation from the small form (elementary body) to the large and back again to the small form The elementary bodies stain red or blue with Giemsa depending on their age They occur in clumps throughout the cell, or they may completely fill the cell cytoplasm They are embedded in a carbohydrate matrix, which can be stained by iodine, but is not always visible in films stained by Giemsa The inclusions found in stained films may be of either or both types

Filtration experiments were inconclusive for many years until Thygeson and his colleagues inoculated a human volunteer with a gradocol filtrate, and thus proved the cause of the disease to be filtrable Elementary bodies were present in the material to be filtered, in the centrifuged deposit of the filtrate and later in conjunctival scrapings from the experimental subject after the onset of symptoms

Further evidence that the elementary bodies are the cause of trachoma was brought forward when it was shown that if

carefully looked for, they can be found in 100 per cent of cases in the early stages They are morphologically indistinguishable from those of inclusion conjunctivitis and of the lymphogranuloma—psittacosis—pneumonitis group to which trachoma virus is closely related

The properties of the virus have not yet been fully investigated due to the lack of a suitable experimental animal apart from the monkey The particle size has been determined, on the basis of direct microscopical measurement of the elementary body to be between 0 2 μ and 0 25 μ with the larger forms varying in size up to 1 μ or more The virus *particles stain readily with Giemsa or with Casteneda stains*

The virus dies rapidly outside the body and as yet no method has been successful in preserving the infectivity of trachomatous material for any length of time Infectivity persists for two weeks in 50 per cent glycerol It is rapidly inactivated by acid or alkali and is destroyed by various chemicals including cocaine which may be important in ophthalmological practice

The only experimental animals of any value are apes baboons and monkeys and their value is limited as they do not develop the typical changes which are seen in human cases They develop instead a follicular conjunctivitis which is clinically identical with a naturally occurring spontaneous folliculosis Inclusion bodies are found only in the ape Nevertheless although the clinical signs are different these animals apparently do develop trachoma as human material passed through successive animals was still capable of causing trachoma when re inoculated into humans

Recently successful isolation of the virus in the chick embryo has been reported but confirmation is still awaited

Relationship to Other Viruses

It has already been seen that the elementary bodies of trachoma are identical microscopically with lymphogranuloma and psittacosis viruses In addition sera from cases of trachoma have been reported to give a positive complement

signs gradually diminish over a period of months but it may take twelve months before the conjunctiva returns to normal

Adults

The onset is less abrupt and the clinical course is milder and more prolonged Follicle formation is prominent but it is confined to the lower lid Enlargement and tenderness of the pre auricular glands is common Although clinically similar to trachoma in its early stages scarring or pannus never occurs

Diagnosis

Inclusion bodies are found in both infants and adults by [illegible] the labora [illegible] are morpho

The delayed onset and negative bacteriological findings are suggestive in the infant and the presence of inclusions is diagnostic as trachoma does not occur in the neonatal stage

In adults the finding of elementary or initial bodies out rules the other forms of follicular conjunctivitis The absence of corneal changes and the swelling of the pre auricular glands distinguishes the condition from trachoma

Treatment

Sulphonamides locally in the infant and orally in the adult, appear to be specific penicillin is less effective if indeed it has any effect Silver nitrate used either prophylactically or therapeutically is of no value Aureomycin is said to give good results

Epidemiology

A reservoir of the virus has been shown to exist in the adult genito-urinary tract In the male the virus gives rise to a non specific urethritis which without treatment runs a chronic course for a period of months before subsiding spontaneously Inclusion bodies can be found in urethral scrapings In the female a cervicitis is caused which gives rise to no

clinical symptoms, and is limited to a ring of transitional epithelium at the external os of the cervix Scrapings from this area will show the presence of inclusion bodies

The condition is widespread and cases have been reported from many areas of Europe, America and Australia It has been found in infants in London, but in a search for a reservoir there it was concluded that the disease was rare—at least amongst those attending V D clinics

Spread

Infants are infected at birth by the mother Spread amongst adults is usually venereal, but the disease may also be contracted by swimming in infected swimming pools These are contaminated by the urine of infected persons

EPIDEMIC KERATO-CONJUNCTIVITIS

Clinical Features

After an incubation period averaging one week there is a sudden onset of conjunctivitis in one eye, which is followed by enlargement and tenderness of the pre auricular gland on the same side Within a week the other eye frequently becomes involved Oedema and congestion of the eyelid and surrounding tissues and of the conjunctivæ are found on examination Two to four weeks after the onset greyish opacities develop in the cornea In very severe cases these may ulcerate, but this is rare While sequelæ are uncommon, the condition lasts for about five weeks during which the patient is more or less disabled Keratitis may also persist in a small proportion of cases

The conjunctival exudate is serous, and it contains no micro organisms until secondary infection sets in during the second week

Diagnosis

Inclusion bodies are not found The exudate or pieces of corneal tissues should be inoculated intracerebrally into mice, into tissue cultures or into chick embryos

Treatment

There is no specific treatment, but convalescent serum has been reported to be of some value

Epidemiology

Cases of the disease have been reported from many countries but the principal incidence is in the United States and the Far East A large number of these cases have occurred in industrial plants and it seems to be most common amongst workers in shipyards

The condition is spread by direct contact with conjunctival exudate or by fomites such as contaminated towels Minor corneal injuries probably predispose to infection

Ætiology

It has been suggested that herpes simplex virus is responsible for many cases, but the clinical features of ophthalmic infection with herpes are readily distinguishable from those of epidemic kerato conjunctivitis

A virus was isolated by Sanders and his colleagues from American cases in one outbreak by inoculating conjunctival scrapings into tissue cultures Their findings have since been confirmed by other workers

Several other viruses have been recovered from cases of this condition but the only one which appears to be of significance is that described by Sanders This is about 100 mμ in size, and can be grown in mice, rabbits and chick embryos Neutralizing antibodies to it develop during convalescence

Chapter Nineteen

THE COLLECTION OF SPECIMENS FOR LABORATORY DIAGNOSIS

The methods used for the laboratory diagnosis of virus diseases may be divided into those aimed at the isolation of the virus responsible, and those which demonstrate a specific immunological response to it In certain cases, also, one may make a diagnosis from the pathological picture presented by the virus This method may be of considerable value in fatal cases or in some of those virus diseases which produce, for example, a specific skin lesion

Isolation of Virus

Generally speaking, few viruses will survive for any length of time outside the body unless they are kept at low temperatures Where practicable, materials for the isolation of virus, such as sputum blood or tissues, should be forwarded to the laboratory in vacuum flasks containing solid carbon dioxide ("dry ice") Should this not be available ice from a domestic refrigerator may be used, provided it is clearly understood that it is not as efficient as is dry ice

In all cases the specimens should be sent by the quickest possible route and the laboratory warned of their arrival Most diagnostic laboratories will give advice and assistance as to the best materials to collect, and the most suitable time for collecting them They will also, in many instances, supply suitable containers *The more material that is sent, within reason, the better will be the prospects of successful isolation*

Serological Diagnosis

This depends on the demonstration of a *rise in the titre of specific antibodies* during the course of the illness *In many*

virus disease antibodies remain detectable in the blood for a considerable length of time after recovery and no particular titre can be regarded as diagnostic if demonstrated on one specimen only. A positive result in a single specimen may be the reflection of a previous illness and bear no relation to that under investigation. For this reason it is always necessary to send two samples. One known as the *acute phase sample* is collected within the first five days of illness and the second or *convalescent sample* is obtained about the tenth day of the illness. In some special cases which are mentioned below it is advisable to send a third specimen collected about two months after the onset of the disease. If a significant rise in the antibody level can be demonstrated between the acute phase sample and those collected later a diagnosis can be made.

Blood collected for serological tests should be taken into a clean dry tube and not less than 10 ml sent to the laboratory.

Materials for the histological diagnosis of infection should be sent to the laboratory in the ordinary way immersed in some fixative such as formol saline. Once the tissues have been placed in fixative they are of course of no value for isolation purposes.

Influenza

The virus may be isolated during the first four days of the illness but rarely later. The patient is instructed to cough and then to gargle with 10 ml of saline to which is then added 5 ml of broth and transmitted to the laboratory. The specimens must be frozen immediately and kept frozen during transit. Suitable containers and flasks may be obtained from the laboratory concerned.

Acute and convalescent stage sera should also be sent for hæmagglutination inhibition and complement fixation tests.

Virus Pneumonia

In the acute stage psittacosis virus may be isolated from sputum, throat washings and the blood or at post mortem

from the lungs and spleen Similarly in cases of Q fever the rickettsiæ should be sought in the blood To complete the investigation of the case infection with influenza virus should also be outruled

Acute and convalescent stage sera should also be collected Any of the following tests may then be performed —

Influenza complement fixation test
Influenza hæmagglutination inhibition test
Psittacosis complement fixation test
Q fever complement fixation test

Cold agglutinins and agglutinins for streptococcus MG are not at their peak until three weeks after onset so that should the above tests be negative another specimen of blood should be collected at this time

Blood intended for cold agglutination test *must not be refrigerated* until the serum has been removed from the clot Otherwise the cold agglutinins will become adsorbed on to the erythrocytes with a consequent drop in titre

Measles

No specific laboratory aids to diagnosis are available

Rubella

No specific laboratory aids to diagnosis are available

Varicella

Vesicle fluid may be collected in capillary tubes and forwarded for microscopical and isolation tests In practice the elementary bodies of varicella are difficult to stain and are few in number when examined under the microscope Similarly varicella virus has not yet been isolated and the laboratory tests for the disease are only of negative value in outruling variola in which both microscopical and isolation tests will be positive

Mumps

The virus may be isolated from the saliva in typical cases, or from the cerebrospinal fluid in cases of meningitis Specimens of saliva should be collected during the first three days of illness

Acute and convalescent stage sera should also be collected for either complement fixation or hæmagglutination inhibition tests The former appears to give more satisfactory results

Herpes Zoster

No specific aids to laboratory diagnosis are available

Herpes Simplex

The virus may be isolated from either primary or recurrent cases Depending on the site and character of the lesion, vesicle fluid mouth swabs or cerebrospinal fluid should be collected Unlike variola it cannot be isolated in the crusting stage

For the serological diagnosis of primary cases acute and convalescent phase sera should be forwarded

An alternative method of diagnosis is by biopsy of a vesicle The best fixative for this purpose is Bouin's fluid

Variola

Scrapings from the prodromal rash or the maculo papular eruption should be collected The lesions are cleaned with ether or spirit, the superficial epithelium is removed and the base scraped with a sharp scalpel The material from each is carefully spread in the centre of a clean glass microscopical slide which is allowed to dry in the air Not less than six such specimens should be obtained The slides are then packed so that they are not in contact with each other, and sent to the laboratory A specimen of blood in a sterile tube should also be taken for isolation purposes Vesicle or pustule fluid may be collected in capillary tubes or on throat swabs If capillary

days or weeks afterwards The length of its presence there varies from patient to patient, and specimens should be collected as early as possible

During an epidemic the isolation of virus from the fæces is not diagnostic, and acute and convalescent sera should also be forwarded for neutralization tests

The virus is not present at any stage in the cerebrospinal fluid

Lymphocytic Choriomeningitis

During the acute stage the virus may be isolated from the blood, cerebrospinal fluid or urine

For serological tests *three* specimens of blood may be collected As well as the ordinary acute and convalescent sera a third specimen should be taken about two months after the onset of the disease

A high lymphocyte count, with no bacteria, is found on cytological examination of the cerebrospinal fluid

Encephalitis Lethargica

No specific laboratory aids to diagnosis are available

St Louis Encephalitis

After death, suspensions of the brain and cord may be inoculated into mice for the isolation of virus The autopsy should be carried out as soon as possible

For serological diagnosis it is best to collect three specimens of blood viz acute, convalescent and after two months

Japanese B Encephalitis

The virus may be isolated from the blood or cerebrospinal fluid, or after death from the brain

Three specimens of blood—acute, convalescent and two months after onset—should be collected

Equine Encephalomyelitis

The western virus may occasionally be isolated from the blood and the cerebrospinal fluid in the acute phase, but the

eastern has never been found in either situation Both eastern and western viruses may be recovered from the brain and cord after death

For serological diagnosis the three specimens of blood should be taken at the times already stated

Louping Ill

The virus has been recovered from the cerebrospinal fluid and from the blood in the acute phase Specimens should be taken for serological diagnosis as for the other viral encephalitides

Russian Spring-Summer Encephalitis

The blood and cerebrospinal fluid contain the virus during the acute phase Three specimens should be taken for serological diagnosis

Note The three specimens of blood, rather than two, are taken because the antibodies to the different encephalitides appear at different times, and in order to cover all cases it may be necessary to have a specimen taken two months after the onset of the illness It is obvious that a knowledge of the encephalitic viruses present in any locality will influence the nature and number of the specimens collected

Trachoma and Inclusion Conjunctivitis

Scrapings should be taken from the conjunctival epithelium after anæsthesia, and spread on clean glass microscopical slides These should be fixed in absolute alcohol for five minutes, carefully packed, and sent to the laboratory

In suspected cases of trachoma the scrapings should be taken from the upper lid Inclusion conjunctivitis primarily affects the lower lid and the material should be collected from there

Epidemic Kerato-conjunctivitis

The virus may be isolated from the exudate or from fragments of corneal tissue

Lymphogranuloma Inguinale

The virus may be isolated from bubo pus, lymph glands or tissue from the affected area In cases of meningo encephalitis it may be recovered from the cerebrospinal fluid The material selected should be as free as possible from secondary bacterial contamination

As the onset of the disease is clinically so insidious it is quite possible that antibodies would be already present before infection were suspected Usually, therefore, only a convalescent phase specimen is available for examination

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